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**PROCEEDINGS OF THE  
1988 SUGAR PROCESSING  
RESEARCH CONFERENCE**

**SEPTEMBER 25-27, 1988  
NEW ORLEANS, LOUISIANA**





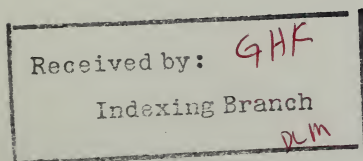
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**Sponsored by  
Sugar Processing Research, Inc.**

**and**

**Southern Regional Research Center  
U.S. Department of Agriculture**

**October, 1989**





## PREFACE

The 1988 Sugar Processing Research Conference is one of a series of Conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference was sponsored by Sugar Processing Research, Inc., and Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture.

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Margaret A. Clarke  
Managing Director  
Sugar Processing Research, Inc.

Stanley E. Bichsel  
President  
Sugar Processing Research, Inc.



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## SUCROSE, SWEETNESS AND SUCRALOSE

Leslie Hough

Department of Chemistry, King's College London, Kensington Campus

Sucrose (1), the most abundant of all crystalline organics, is an attractive feed stock for chemical and microbiological exploitation, with potential applications in the chemical, pharmaceutical, food and related industries. The chemistry (Jenner, 1980) of this unique, non-reducing disaccharide is determined by the multiplicity of hydroxyl groups, eight in all, and the acid labile inter-glycosidic bond joining the  $\alpha$ -D-glucopyranosyl and  $\beta$ -D-fructofuranosyl units.

The first synthesis of sucrose (1) by Hassid and Doudoroff in 1944 used enzymes, followed in 1953 by an ingenious chemical synthesis from D-glucose and D-fructose derivatives by Lemieux and Huber (1953). A similar chemical synthesis (Queen's Univ., 1979) of the unnatural L-sucrose (2), the mirror image of natural D-sucrose by combination of L-glucose and L-fructose derivatives, led to the surprising discovery that it was sweet. The sweetness of L-glucose and L-fructose has been known for many years (Shallenberger, 1973), in fact ever since Emil Fischer's pioneering studies on the carbohydrates and amino acids.

The L-sugars are of considerable interest (Bakai, 1984) since these unnatural but sweet sugars are not metabolised; hence they have potential as non-nutritive sweeteners but with the proviso that they could be manufactured economically. The absorption of L-glucose and L-fructose into the blood stream and circulation for long periods could prevent their use. The equivalent sweetness of D- and L-sucrose contrasts with D- and L-amino acids, since the D-forms are usually sweet whereas the L-forms are not (Kier, 1972; Fischer, 1980). For example, D-tryptophan (3) is 35 times sweeter than sucrose, whereas L-tryptophan is bitter, and D-phenylalanine (4) is seven times sweeter but the L-isomer is bitter. It should be noted that in the D- $\alpha$ -amino acids, the amino ( $\text{NH}_2$ ), carboxylic acid ( $\text{CO}_2\text{H}$ ) and side chains (R) are oriented in a clockwise (R) configuration whilst these groups are in the opposite, anti-clockwise configuration (S) in the L-isomers. These differences led Louis Pasteur to postulate that the taste bud receptors are asymmetric or chiral; consequently mirror image compounds of sweet substances should give different responses (Shallenberger, 1971). The similarity in the sweetness of D- and L-sugars would therefore appear to be anomalous.



Sweetness is also induced by a surprising variety of remarkably different chemical structures, compounds such as saccharin (5), cyclamate (6), acesulpham-K (7) and neohesperidin dihydrochalcone (8), which are all artificial or synthetic and were discovered by serendipity because of their intense sweetness. Deutsch and Hansch (1966) proposed that sweet compounds functioned in the taste bud by interaction with an area of hydrophobic bonding that is coupled with a site for electronic bonding, similar to the mode of action of drugs.

Shallenberger and Acree (1967, 1969) focused attention on a structural feature that all sweeteners have in common, namely two constituent electronegative atoms, A and B, separated by 2.5-4.0Å (or 260-300pm), with a hydrogen atom linked covalently to A (2), as in 5 through 8. The glycol group is the minimum requirement for sweetness in the sugars; consequently, a pair of hydroxyls on adjacent carbon atoms was designated as the AH/B unit, with one hydroxyl functioning as the AH and the oxygen atom of the other hydroxyl as the B (10). It was postulated that the sweetness sensation arose by the creation of a pair of hydrogen bonds from this AH/B unit to another AH/B unit but of opposite configuration on the proteinaceous receptor site.

Proteins are ideally suited to form hydrogen bonds, since the peptide bonds have amide (N-H) and carbonyl (C=O) groups, which could act as the reciprocal AH/B units (2,10). In the case of sugars, A and B are the same, namely oxygen atoms; hence either one of the two hydroxyls could act as the AH or B. It follows they could interchange their AH/B role, but in all other types of sweet compounds, including the amino acids, this is impossible. The difference in the behaviour of sweetness of the D- and L- forms of amino acids and of sugars (Shallenberger, 1973) could therefore be due to the interchangeable AH/B unit in the sugars. Whilst the Shallenberger hypothesis (Shallenberger & Acree, 1967, 1979) rationalised the characteristic structural element in all sweet compounds, it was recognised that a vast number of organics also have identifiable AH/B units (Bragg et al., 1978) but which are not sweet. Additional criteria were sought in order to refine the structural requirements for sweetness to occur. Another parameter emerged from a study by Kier (1972) of 1-alkoxy-2-amino-4-nitrobenzenes (11), namely a third group X which is hydrophobic (lipophilic) in nature and serves to guide or adsorb the sweet compound to the taste receptor. This additional criterion gives a triangle of groups (Shallenberger and Lindley, 1977a, 1977b; Heijden et al., 1978) (X, AH and B) known as the glucophore (12). The lipophile-hydrophile balance is an important factor in sweetness intensification, and all compounds (e.g. 3-8) that are sweeter than sucrose are more hydrophobic. Sugars are hydrophilic water-loving molecules that are only weakly attracted to the taste buds, and hence not very sweet in comparison with, for example, saccharin (5).



Small changes in a monosaccharide structure can have a major influence upon its sweetness; thus D-glucose is significantly sweeter than D-galactose, its carbon-4 epimer, and D-fructose (13), the 5-epimer of L-sorbose is five times as sweet (Shallenberger, 1973). D-Fructose (13), a ketose, is somewhat sweeter than D-glucose, its aldose epimer, and fructose is also sweeter than sucrose (1), and this is due largely, if not entirely, to the high sweetness of  $\beta$ -D-fructopyranose (13) (Table 1).

Table 1.--The sweetness of compounds related to sucrose.

	Sweetness
Sucrose ( <u>1</u> )	1.0
<u>D</u> -Glucose*	0.6-0.75
<u>D</u> -Galactose*	0.4-0.5
<u>D</u> -Fructose*	1.1
$\beta$ - <u>D</u> -Fructopyranose ( <u>13</u> )	1.8
Methyl $\alpha$ - <u>D</u> -Glucopyranoside ( <u>17</u> )	0.1
Trehalose ( <u>18</u> )	0.1
Methyl $\beta$ - <u>D</u> -Fructofuranoside ( <u>19</u> )	0
$\beta$ - <u>D</u> -Glucopyranose ( <u>16</u> )	0.6-0.75
* mutarotation equilibrated	

Consequently, we have a fourth factor to consider in predicting or accounting for the sweetness of organic compounds, namely the overall shape or conformation of the molecule coupled with the configuration at individual atoms and their effect upon the conformation.  $\beta$ -D-Fructopyranose adopts a chair conformation (13) (Shallenberger & Acree, 1969; Lindley & Birch, 1975; Shallenberger, 1978; Birch & Mylvaganam, 1976) in which only one glucophore triad is possible, with AH=2-OH, B=1-O and X=6-H's and when viewed along the C<sub>2</sub>-C<sub>3</sub> bond, the AH/B/X triad has a clockwise orientation. Methyl  $\beta$ -D-fructopyranoside with the anomeric 2-OH blocked is much less sweet than  $\beta$ -D-fructopyranose and the furanoside is not sweet at all.

The sweetness of L-fructose (14) can be explained by reversing the role of the hydroxyls to give the same clockwise orientation of the glucophore (AH=1-OH, B=2-O and X=6-H's) (14). If the taste receptor is chiral, the clockwise glucophore allocated to D-fructose and L-fructose should also arise in the D-amino acids, and it does with AH=NH<sub>2</sub>, B=CO<sub>2</sub>H and X=R completing a clockwise triad (15). In the L-amino acids the arrangement of these groups is anti-clockwise, which suggests that this gives rise to the bitter taste in organic compounds.

When the ring-oxygen of a pyranoid or furanoid sugar is replaced by a methylene group, it is known as a pseudo-sugar, for example,  $\Psi$ - $\beta$ -D-glucopyranose (16). Since  $\Psi$ - $\beta$ -D-glucopyranose,  $\Psi$ - $\alpha$ -D-galactopyranose and  $\Psi$ - $\beta$ -D-fructopyranose are similar in sweetness to their parent sugars (Suami, 1987), it follows that the ring-oxygen has little influence in determining the sweetness of sugars.

The sweetness of sucrose cannot be due to the individual glucosyl and fructosyl units since these two components of the disaccharide, as represented by methyl  $\alpha$ -D-glucopyranoside (17), or trehalose (Birch, 1976) (18), and methyl  $\beta$ -D-fructofuranoside (Shallenberger, 1978) (19), are one tenth as sweet and zero respectively (Table 1). These observations suggested a special or unique arrangement (Hough, 1985) of the glucophore in sucrose wherein a hydroxyl from each unit or each half of the disaccharide participates in the AH/B group. In the crystalline form, sucrose adopts a conformation (Jenner, 1980) with the  $\alpha$ -D-glucopyranosyl unit in a chair form ( $^4C_1$ ) and the  $\beta$ -D-fructofuranoside unit in a twist shape ( $^4T^+$ ), and there are two intramolecular hydrogen bonds bridging from O-6' to O-5 and from O-1' to O-2 which hold the furanose ring at right angles to the plane of the pyranose ring. N.m.r. studies (Bock & Lemieux, 1982; Christofides & Davies, 1985, 1987) of sucrose in solution (deuterated dimethyl sulphoxide) have detected two hydrogen bonded conformations (20 and 21) in equilibrium, due to the 2-hydroxyl of the glucosyl unit acting as a hydrogen bond acceptor for either the 1'-hydroxyl (21) or the 3'-hydroxyl (22) of the fructofuranoside unit, and in the approximate ratio of 2:1 respectively.

Examination of molecular models of the conformations of sucrose to find those groups of atoms that coincide with the approximate dimensions of the Kier-Shallenberger triangular template (Lee, 1987a) suggested that two such glucophores, one involving X=4-H, AH=1'-OH and B=2-O (23), and the other X=4-H, AH=3'-OH and B=2-O (22). Both of these triads are clockwise arrangements in agreement with the aforementioned chiral receptor theory. Support for these assignments (Hough, 1985) came from the synthesis of galacto-sucrose (24) by inversion of the configuration at C-4 of sucrose (Chowdhary et al., 1984), which resulted in loss of sweetness (Lindley et al., 1976). On the other hand 4-deoxy sucrose (25) retained its sweetness. Hence the axial configuration of the hydrophilic 4-hydroxyl of galacto-sucrose (24) cannot be tolerated, and the equatorial 4-hydroxyl in sucrose is not essential for sweetness. Galacto-sucrose (24) and 4-deoxysucrose (25) were conveniently synthesized (Chowdhary et al., 1984) by conventional methods from the readily available heptapivalate (26) (Scheme 1). Selective esterification of sucrose with pivaloyl chloride (2,2-dimethylpropanoyl chloride) provides a valuable route to specifically blocked esters, many crystalline, such as the 6,6'-di-ester, 1',6,6'-tri-ester, 1',4',6,6'-tetra-ester and 1',2,4',6,6'-penta-ester, all in >30% yield (Scheme 1) (Chowdhary et al., 1984).

The configuration of the 3-hydroxyl of sucrose must be equatorial for sweetness in sucrose since its 3-epimer, allo-sucrose (28) (Hough & O'Brien, 1980), proved to be tasteless, possibly because the large 3-hydroxyl replaces the small axial 3-hydrogen substituent in sucrose, thereby exceeding the restricted area at this site on the receptor of the taste bud. allo-Sucrose (28) was prepared (Hough & O'Brien, 1980) from sucrose by oxidation with either Agrobacterium tumefaciens or dimethyl sulphoxide-acetic anhydride, to give 3-keto-sucrose (27), which on reduction with sodium borohydride, afforded allo-sucrose (28) and sucrose (1) in the ratio

12:1. Hence by blocking, modifying or replacing specific hydroxyls in sucrose (Lee, 1987a; Hough, 1985) it should be possible to locate the hydroxyls that are responsible for the sweet taste and provide an outline of structure versus sweetness activity (Hough & Khan, 1978).

As illustrated above, esterification of sucrose can be highly selective, yielding under controlled conditions mono- to the fully substituted octa-esters. There are 8 possible mono-esters and only one octa-ester, but in theory 28 di- and 56 tri-esters could arise (Hough, 1985). Fortunately higher reactivity at the primary hydroxyls, C-6, C-1' and C-6', simplifies these reactions, the order of reactivity being  $\text{HO-6, HO-6'} > \text{HO-1'} > \text{HO-2}$ . The primary 1'-hydroxyl is less reactive than those at the 6- and 6'-positions because of its hindered, neopentyl character. Mono-esterification of sucrose has a remarkable effect since its 6-mono-acetate (Kononenko & Kestenbaum, 1961; Khan, unpublished data) (28:  $\text{R-O.CO.CH}_3$ ) is only slightly sweet and its 6-benzoate (29:  $\text{R-O.CO.Ph}$ ) and 6-phosphate (29:  $\text{R-O.PO}_3\text{H}_2$ ) are not sweet.

Large groups at carbon-6 appear to result in loss of sweetness due to a misfit of the enlarged molecule on the receptor site, since smaller groups at carbon-6 do not have a significant effect on sweetness, as in 6-deoxy-sucrose (29:  $\text{R-H}$ ) and 6-O-methyl sucrose (29:  $\text{R-OCH}_3$ ) (Lindley et al., 1975, 1976). Significantly, the larger 6-O-benzyl ether (29:  $\text{R-O.CH}_2\text{Ph}$ ) is not sweet. The sweetness of 1'-deoxy- and 1'-O-methyl-sucrose favour the participation of triangle of groups at C-4(X), C-2(B) and C-3'(AH) (22).

1'-Deoxysucrose (33) was synthesised by reductive dehalogenation of 1'-chloro-1'-deoxysucrose (32) which can be obtained from the 1',6,6'-tri-trimsylate of sucrose (30) by benzoate displacement of the trimesitylene sulphonyloxy groups at C-6 and C-6', giving the 1'-trimsylate of the 6,6'-dibenzoate (31) followed by chloride displacement of the 1'-trimesitylene sulphonyloxy group (Guthrie & Watters, 1980). The 1'-chloro group of 32 had a significant effect upon the rate of acid hydrolysis which was reduced when compared with sucrose by 10 times, and also it inhibited the hydrolytic action of invertase, a  $\beta$ -D-fructofuranosidase. In addition, loss of sweetness was observed when the 3'-hydroxyl was esterified, as in 3'-O-acetylsucrose (James, 1988) (34), presumably by eliminating the AH of the glucophore.

Enhancement of the sweetness of sucrose would appear to require derivatisation by lipophilic groups especially at the axial 4-position (Hough & Khan, 1978) and possibly the 1'-position, but the hydroxyls at C-2 and at C-3' must remain unsubstituted since they appear to be essential components of the glucophore (22). Aspartame (35) ('NutraSweet') or L-aspartyl-L-phenylalanine-methyl ester (Crammer & Ikan, 1977) requires the ester since the free acid is tasteless; and of its four isomers only the L,L-isomer is sweet (100-200); the D,D-D,L- and D,D-isomers are tasteless (Mazur, 1976). The introduction of a fenchyl group (Fukino et al., 1973) (35)



enhances its lipophilicity, which results in the sweetness increasing enormously to over 100 fold. When D-tryptophan (35x) was converted into its 6-chloro-derivative (36) (1000x) its sweetness increased by 30 fold (Crammer & Ikan, 1977), but a related chlorination of saccharin (600-700x) to give 6-chlorosaccharin (37) (100-350x) was not so successful (Crammer & Ikan, 1977). Any intensification of the sweetness of sucrose would clearly reduce caloric intake from this source and derivatisation could also inhibit metabolism by blocking the hydrolysis action of invertase and  $\alpha$ -glucosidase.

Chloro derivatives of sucrose were available from sucro-chemical studies and had been developed as convenient intermediates for the synthesis of thio-, deoxy- and amino-deoxy sugars, rare sugars with potential applications in the biological fields (Szarak, 1973).

The direct replacement of a hydroxyl group in a carbohydrate by a chloro substituent was discovered by Helferich and his colleagues (1921, 1923, 1925) when they reacted methyl  $\alpha$ -D-glucopyranoside (17) with sulphuryl chloride ( $\text{SO}_2\text{Cl}_2$ ) in pyridine-chloroform at 5° and obtained a 4,6-dichloro-4,6-dideoxy-2,3-cyclic sulphate derivative. J.K.N. Jones and his co-workers (Bragg et al., 1959; Jones et al., 1960) showed that the reaction proceeded with inversion of chirality at carbon-4 to give a galactoside, methyl 4,6-dichloro-4,6-dideoxy- $\alpha$ -D-galactopyranoside 2,3-cyclic sulphate (41).

Application of this reaction to sucrose by Bragg, Jones and Turner (Bragg et al., 1959) gave rise to a complex mixture of chloro derivatives, in which the glucosyl residue was similarly converted into a 4,6-dichloro galactosyl derivative but the fructosyl unit had presumably undergone varied reactions. Repetition of the reaction at 50° gave, after separation by chromatography, a tetrachloro and two pentachloro derivatives of sucrose in which the fructosyl unit had been converted into a 3',4'-epoxide (42), a 3'-ene (44) and a 1',4',6'-trichloride (45) (Ballard et al., 1973).

Cyclic sulphate formation can be avoided by using the minimum quantity of pyridine in the sulphuryl chloride reaction when chlorosulphate esters are then formed; and any that remain after chlorination can be removed by treatment with methanolic sodium iodide solution, thereby liberating the hydroxyl groups (Jennings and Jones, 1963). This variation of the reaction with methyl  $\alpha$ -D-glucopyranoside (17) then gave methyl 4,6-dichloro-,6-dideoxy- $\alpha$ -D-galactopyranoside (42). Study of the progress of this reaction at low temperature showed that the initial product was the 2,3,4,6-tetrachlorosulphate (38), which then underwent nucleophilic bimolecular substitution by chloride anions at the more reactive primary 6-position, giving the 6-chloride (39), followed by a slower reaction at the secondary 4-position, in this case accompanied by inversion of configuration, to form the final product, the 4,6-dichloro-galactoside 2,3-bis-chlorosulphate (40). Substitution reactions at carbon-2 and -3 are inhibited by unfavourable transition states (Szarak, 1973), and the chlorosulphate groups can then be removed to give (42).

A similar approach was used to study the reactions of sucrose with sulphuryl chloride, and the major pathway (Hough, 1985) proceeded first to the 1'-chloride (46) (29% yield) then to the 6,6'-dichloride (47) (29% yield) (Ballard et al., 1973) and progressed via the 4,6,6'-trichloro-galacto-sucrose (48) (50% yield) (Hough et al., 1975a; Parolis, 1976) to the 4,6,1',6'-tetrachloro (49) (45% yield) (Lee, 1987b) and 4,6,1',4',6'-pentachloro-derivatives (50) (Lee, 1987b; Phadnis, unpublished results). The introduction of the 4'-chloro-substituent in the fructosyl unit probably proceeds via a 3',4'-epoxide (43) (Lee, 1987b) since steric factors prevent direct substitution of the 4'-chloro-sulphate. The observed selectivity in the reaction of the sucrose with sulphuryl chloride suggested that the order of reactivity was  $\text{HO-6'} > \text{HO-6} > \text{HO-4} > \text{HO-1'} > \text{HO-4'}$ . The chlorination is slower at the primary 1'-hydroxyl than at the 6- and 6'-positions because it is a hindered neo-pentyl type, adjacent to the 2'-anomeric group. The 6,6'-dichloride (47) can be conveniently prepared (Annisuzzaman & Whistler, 1978, 1980) in high yield (>70%) by a highly selective reaction of sucrose with triphenylphosphine in carbon tetrachloride and pyridine.

Originally 4,6,1',6'-tetrachloro-4,6,1',6'-tetra-deoxy-galacto sucrose (49) was synthesised (Hough & Phadnis, 1976) from the 6,1',6'-trimethylate (30) of sucrose by substitution of the sulphonyloxy groups with lithium chloride to give the 6,1',6'-trichloride (51), which underwent a selective reaction with sulphuryl chloride, as anticipated, at carbon-4 with inversion of configuration. When this compound (49) was tasted, it was 200 times sweeter than its parent, sucrose, the first time that the sweetness of a carbohydrate had been intensified by derivatisation (Hough & Phadnis, 1976). A prior taste study had revealed that a slightly different but similar trehalose derivative, 4,6,4',6'-tetrachloro-4,6,4',6'-tetra-deoxy-galacto- $\alpha,\alpha$ -trehalose (52) was as bitter as quinine (Birch, 1977), thus emphasising the importance of structure and configuration in determining sweetness and bitterness.

This discovery led to the synthesis (Hough et al., 1979) of an extensive range of chloro derivatives of sucrose for pharmacological and toxicological evaluation, and the assessment of their sweetness and quality of taste.

The 1',6'-dichloride (53) was obtained by selective nucleophilic 6-mono-substitution of the 6,1',6'-tri-O-trimethylate (30) (Creasey & Guthrie, 1974; Almquist & Reist, 1976a, 1976b) with benzoate thus giving the 6-benzoate 1',6'-disulphonate, which gave the required 1',6'-dichloride (53) on subsequent reaction with chloride anions (Hough et al., 1975b). 1'-Chloro-1'-deoxy-sucrose (32) was 20 times sweeter than sucrose, similar to the 6'-chloro derivative (46), but 6-chloro-6-deoxy sucrose (54) was bitter and not sweet. On the other hand, the 4-chloro-galacto-sucrose (55) derivative, prepared (Hough et al., 1984) from the readily available heptapivalate (4-OH free: 26), was 5 times sweeter (Hough & Khan, 1978).

Like the 6-chloro derivative (54), 6,6'-dichloro-6,6'-dideoxy sucrose (47) was not sweet, the 6-chloro substituent clearly having an adverse effect due either to the size of the substituent at carbon-6, as described earlier, or to the 6-chloro group locking onto the hydrophobic receptor site to give a misfit (Hough & Khan, 1978). In the 1',6'-dichloro derivative (53), the additive effect of the chloro groups was synergistic, enhancing the sweetness more than 70 times, and this effect was even greater in 4,1'-dichloro-4,1'-dideoxy-galacto-sucrose, (56) with 120 times the sweetness of sucrose. Synthesis of the latter (56) was achieved by direct chlorination of a 6,6'-diester of sucrose with sulphuryl chloride at the 4 and 1' positions (Khan, unpublished data).

Substitution of another chloro substituent in (56) at the 6'-position, to give the 4,1',6'-trichloro derivative (57) enhanced the sweetness five fold, and this product 4,1'-6'-trichloro-4,1',6'-trideoxy-galacto-sucrose ("sucralose") (57) was 650 times sweeter than sucrose (Hough et al., 1979), non-toxic, not-metabolised and hence non-caloric. Sucralose is significantly more stable to acid hydrolysis than sucrose and has been developed jointly by Tate & Lyle plc (U.K.) and Johnson and Johnson (USA) for approval to market as a high quality intense sweetener. Sucralose is synthesised (Fairclough et al., 1975) by formation of the 6,1',6'-tri-O-trityl ether of sucrose (58), followed by acetylation to (59) which on de-tritylation rearranges to give the key 2,3,6,3',4'-penta-acetate (60) intermediate by a 4→6 acetyl migration. Substitution of the free hydroxyls at carbons 1',4 and 6' by chlorination with SO<sub>2</sub>Cl<sub>2</sub>, followed by the removal of the acetate groups by de-esterification, yields the required 4,1',6'-trichloro derivative of galacto-sucrose (57) as a water soluble, white, crystalline solid.

The next development was to synthesise sucrose derivatives with a 4'-chloro substituent on the fructofuranoside (Lee, 1987b; Khan, 1984) by exploiting a novel conversion (Guthrie et al., 1980) of sucrose into its 3',4'-lyxo-epoxide by the agency of triphenylphosphine (T.P.P.)-diethylazodicarboxylate (D.E.A.D.). Application of this reagent to 4,1',6'-trichloro-4,1',6'-trideoxy-galacto-sucrose (sucralose) (57) gave the corresponding lyxo-epoxide (61) which on nucleophilic attack with chloride anion, underwent ring-opening stereospecifically at carbon-4' to revert back to the fructo-configuration with a 4'-chloro group, giving the required 4,1',4',6'-tetrachloride (62). Since this product (62) is 2,200 times sweeter than sucrose the replacement of the 4'-hydroxyl by chloride results in a four-fold increase in the sweetness of sucralose (Lee, 1982, 1987b). The corresponding 4,1',4',6'-tetrabromo derivative has a remarkably high sweetness, 7,500 times that of sucrose. All of the halogeno-substituents in sucralose (57) and its 4'-chloro derivative (62) are on the upper face of the molecule. This is a significant factor since the isomeric sorbo-tetrachloride (64) in which the 4'-chloro group has the opposite configuration, below the plane of the ring, was greatly reduced in



comparison to a sweetness only 200 times sweeter than sucrose. The sorbo-tetrachloride (64) was synthesised via the 3',4'-ribo-epoxide (63) by nucleophilic ring opening, again selectively at carbon-4' with chloride anions (Lee, 1987b).

The 6,1',6'-trichloro derivative of sucrose (51) is 25 times sweeter than sucrose and as we have observed, when the 4-hydroxyl is replaced by a chloro group in the 4,6,1',6'-tetrachloro derivative (49), the sweetness increases to 200 times (Hough & Khan, 1978). However, replacement of the 2-hydroxyl group in 51 by chloride with inversion of configuration to give the 2,6,1',6'-tetrachloro manno-sucrose derivative (Khan and Jenner, 1980), it was as bitter as quinine. This observation supports the view that the equatorial 2-hydroxyl is essential for sweetness, combined with the 3'-hydroxyl, as triangular array of saporophoric groups with lipophilic substituents at specific positions in the molecule, preferably on the upper face (Hough & Khan, 1978).

Similar attempts to enhance the sweetness of other carbohydrates such as D-glucose, maltose and trehalose by the insertion of chloro substituents, as for example, 6-chloro-6-deoxy-D-glucose, 4',6'-dichloro-4',6'-dideoxymaltose and 4,6-dichloro-4,6-dideoxytrehalose, were unsuccessful, the majority of the halogenated derivatives being less sweet than the parent and many bitter (Dziedzic & Birch, 1981). Likewise, 4-chloro-D-galactose and its simple glycoside derivatives (65) are not sweet (Thelwall, unpublished results) emphasising the unique role of the 2- and 3'-hydroxyl groups in the sweetness of the sucrose molecule (22).

The observation that 6-chloro-6-deoxy-D-fructofuranose (66) is sweet (<1x), and the 1,6-dichloro derivative (67) slightly sweeter (1x), was unexpected since the parent D-fructofuranose is, in all probability, not a sweet compound. Whilst they do not compare in sweetness to sucralose (650x), or 1',6'-dichloro-sucrose (80x), this unit (67) was suggested as a possible contributor to the glucophore(s) in sucralose. A detailed investigation of the conformation of crystalline sucralose (57) using X-ray analysis (Kanters et al., 1988) demonstrated an intramolecular hydrogen bond between the 2-hydroxyl and the 3'-hydroxyl (68). Simple <sup>1</sup>H-n.m.r. spectroscopy (Christofides et al., 1986) of four 1'-chloro-1'-deoxysucrose derivatives, including sucralose (57), in dimethyl sulphoxide, revealed the presence of an intramolecular 3'-OH....O-2 hydrogen bond, in which the 3'-OH is the donor and 2-OH is the acceptor hydroxyl group. This hydrogen bond stabilises cooperative bonding in both glucosyl and fructoside units (3-OH ... 2-OH ... 3'-OH ... 4'-OH) (69).

The internally hydrogen bonded conformation (68) of these sucrose derivatives closely resembles the glucophore proposed for sucralose and sucrose (22) and would require the minimum energy to transform the sweetener to the doubly external hydrogen-bonded form attaching the sweet compound to the sweet sensitive protein (12). Suami (1987) has examined molecular models of this type of interaction, assuming that the protein is an  $\alpha$ -helix which is N-terminated by

L-serine or L-threonine, a chiral receptor with NH<sup>+</sup> and OH groups acting as AH and B respectively. The hydrophilic group then appears on the fourth amino acid unit from the N-terminal of the protein. The models show quite clearly that under these circumstances the glucophoric groups (AH/B/X) must be clockwise on the sweet compound to match the anticlockwise array on the receptor protein; otherwise, they do not engage in a three point interaction.

The studies on chloro-sucroses have given some insight into the obscure complexities of the structure-activity relationships amongst sweet molecules, a challenging area of research that will require an understanding of the underlying physiological and neurological mechanisms and responses.

#### ACKNOWLEDGEMENTS

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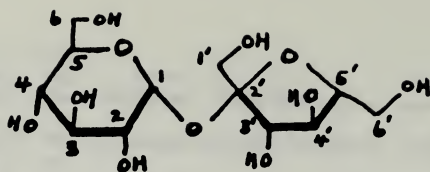
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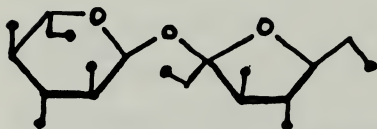
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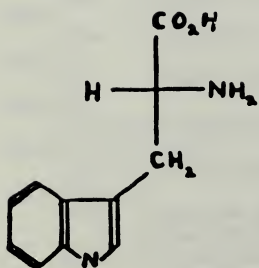
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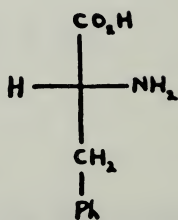
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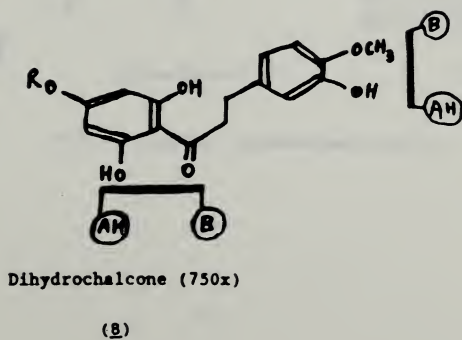
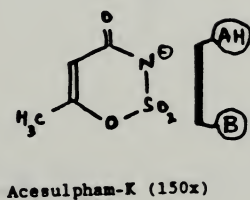
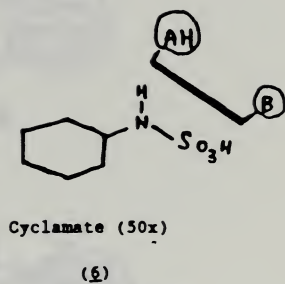
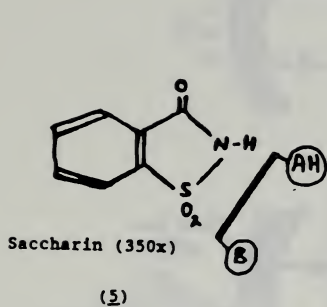
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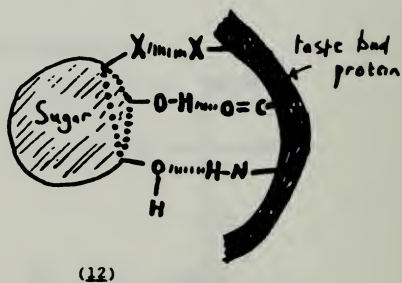
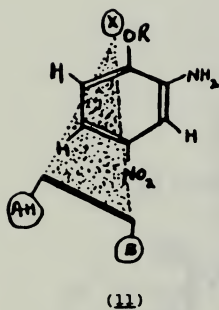
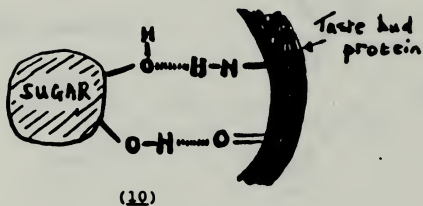
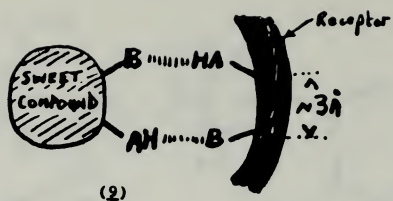


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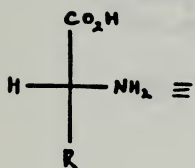
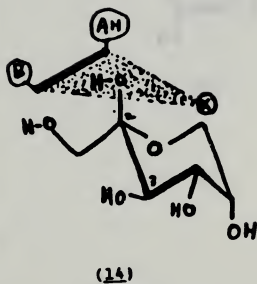
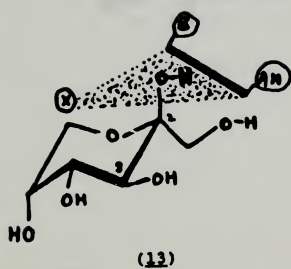


(4)





The Kier-Shallenberger Triangle (AH/B/X)

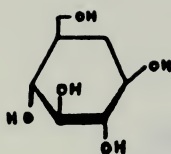


D-amino acid

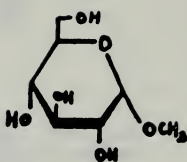


R-configuration

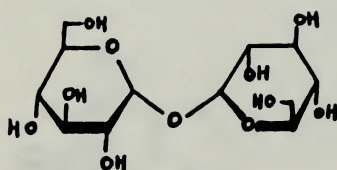




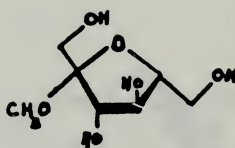
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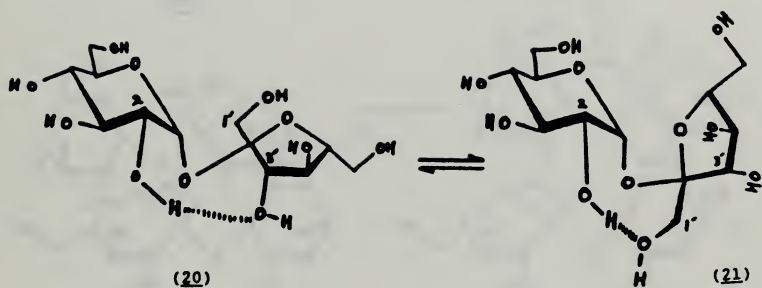


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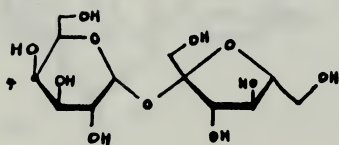




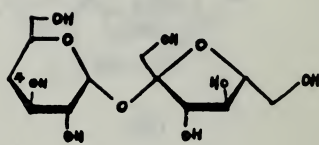
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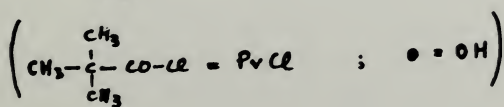
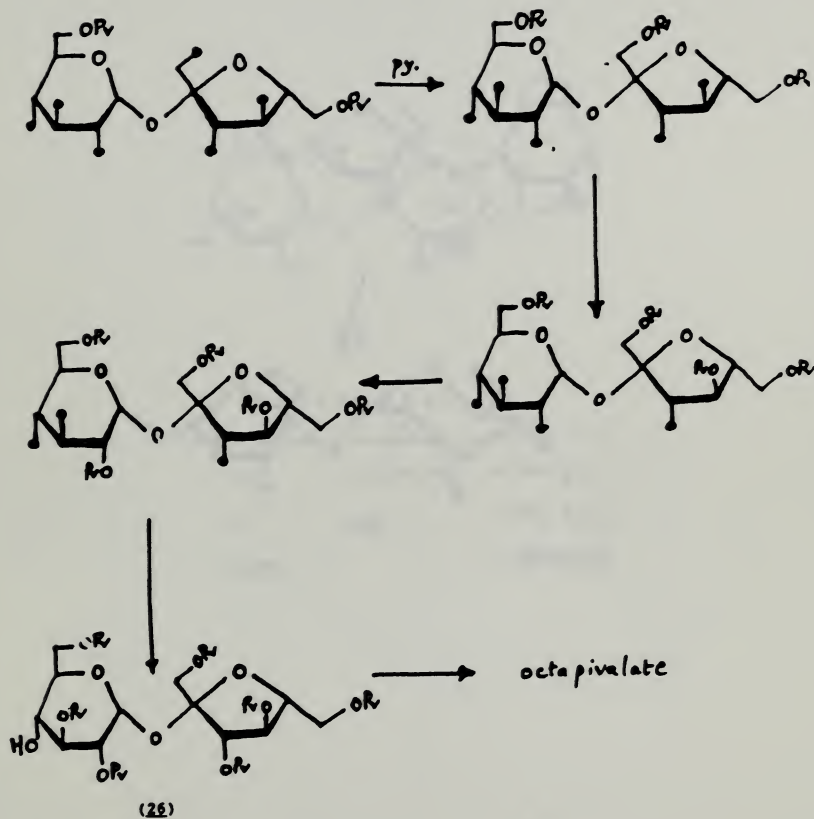


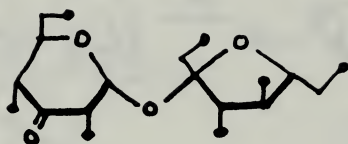
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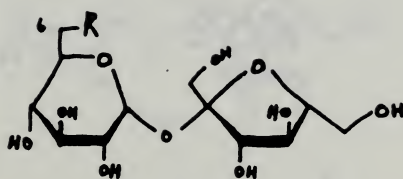


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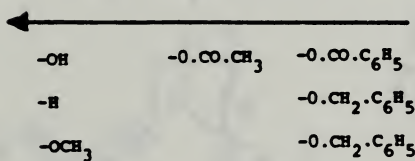
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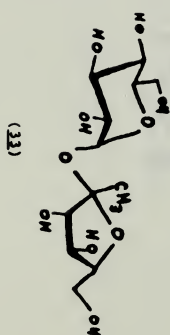
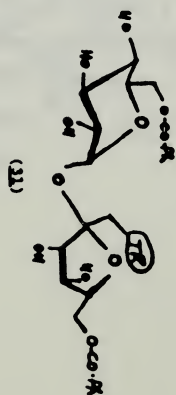
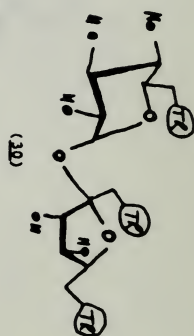
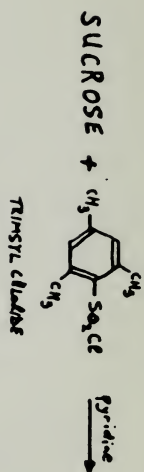
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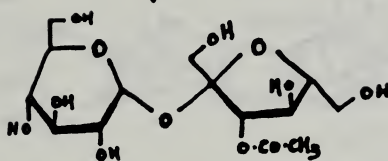
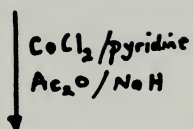
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SWEETNESS

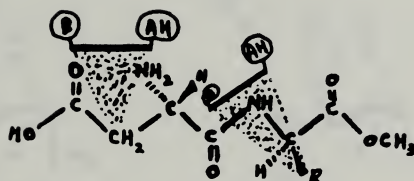




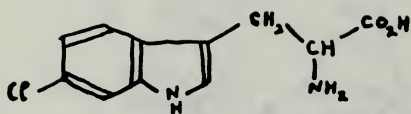
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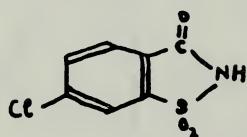
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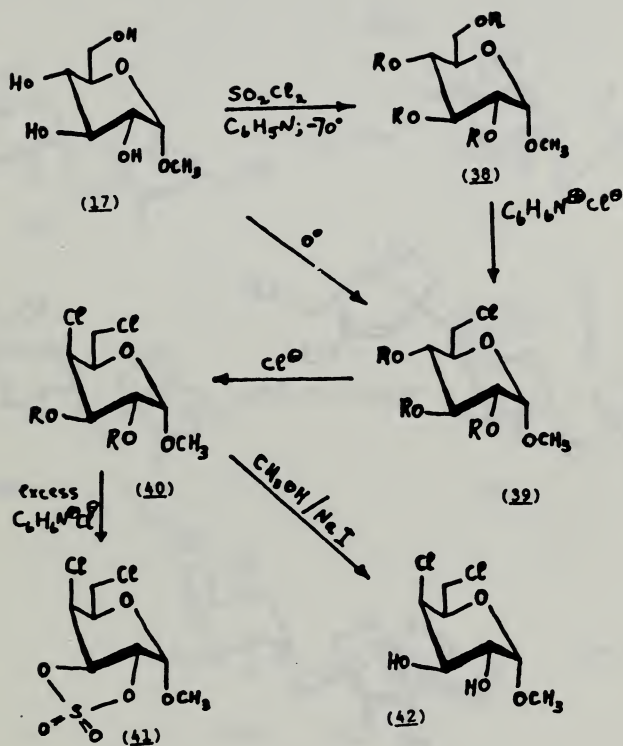


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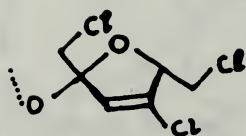
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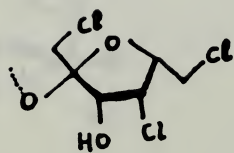




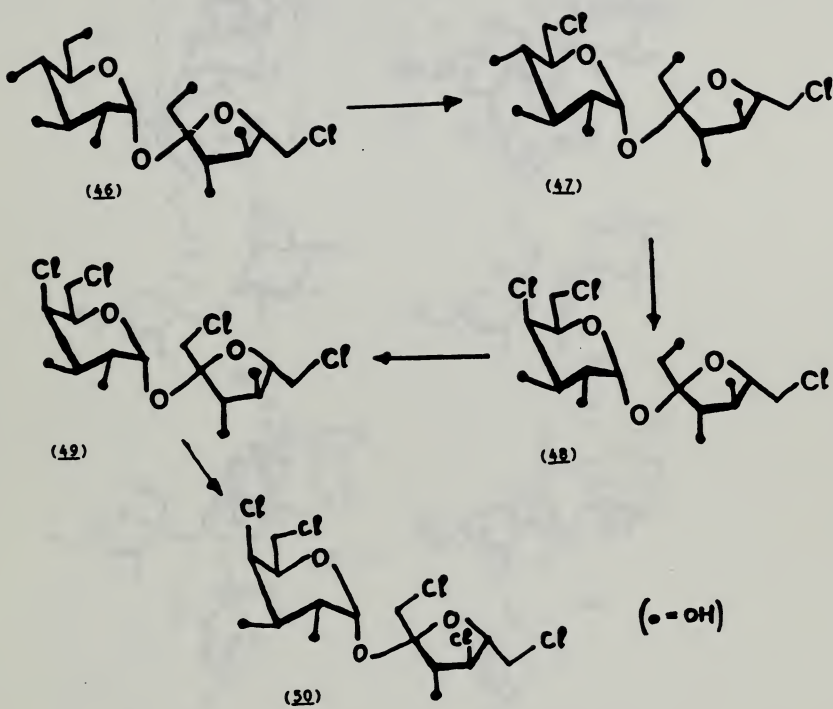
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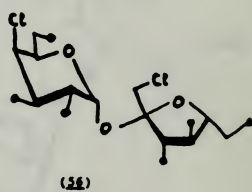
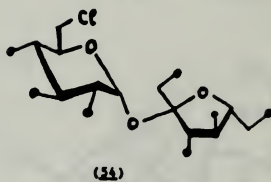
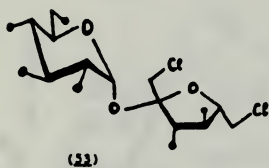
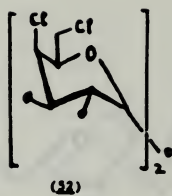
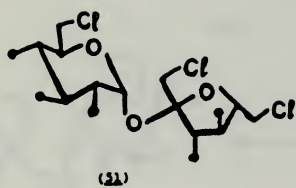


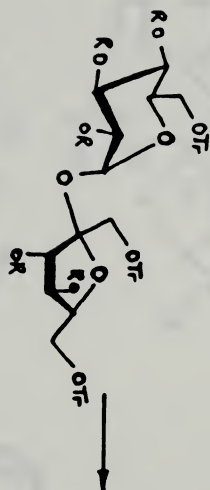
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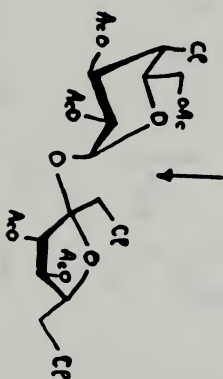
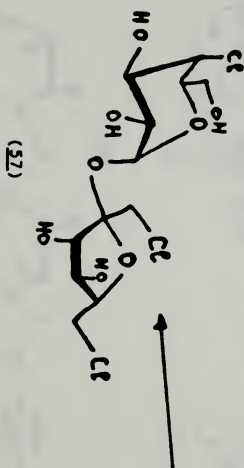
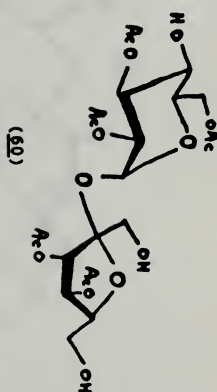
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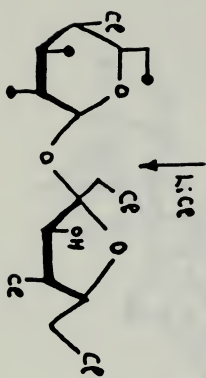
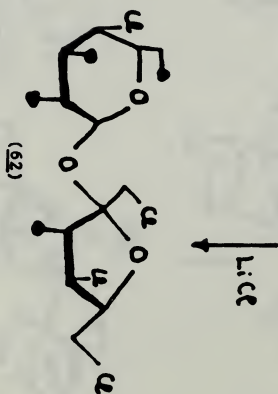
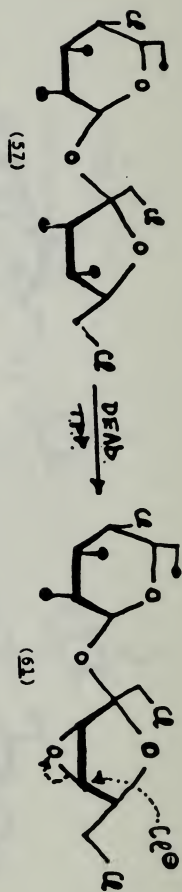


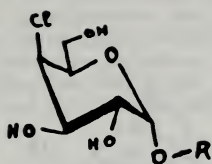




(59: R = CO · CH<sub>3</sub>.)



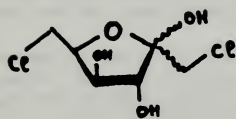




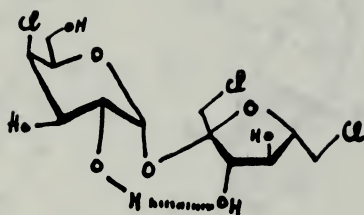
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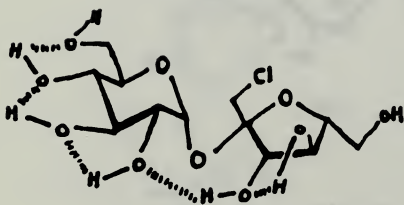
(66)



(67)



(68)



(69)



## DISCUSSION

Question: To make sucralose from sucrose takes five steps. What is the over-all yield?

Hough: I cannot tell you on a per pound basis. However, the tritylation proceeds in greater than 80% yield; the esterification then is pretty well quantitative; the detritylation occurs in greater than 85% yield; the halogenation occurs in approximately 85% yield. Thus, the yield is quite respectable. Of course, the expensive reagent is trityl chloride, which can be recovered. Sulphuryl chloride is cheap, and pyridine is relatively cheap, and can be recovered.

So, once you have (a) a process for synthesizing trityl chloride, and (b) recovering trityl alcohol, then the process should be economical.

Question: The original hypothesis by Fischer seems to have been an empirical one. Has there been any corresponding empirical suggestions as to the nature and cause of bitterness, which might be explained by the structure of some of the compounds you have mentioned, which are extremely bitter?

Hough: I am not an expert on bitterness, and the only comment I can make is the one I made earlier. Namely, that one notices that sweetness is lost when you get away from a clockwise orientation of the AH/B/X groups (and, of course they are clockwise in Sucralose, a point I may not have made earlier). We suspect that with bitterness, the orientation is either not clockwise or else there is some other modification. In certain orientations there is no flavor at all.

Question: The taste buds for bitterness are different from the ones for sweetness--they are not in the same location, so it could be that the receptors are the mirror-image of the sweetness receptors and possibly also better to accommodate larger groups.

Question: Is anything known about the toxicology of these compounds? If they are going to be made commercial, particularly in this country, you will have to pass the FDA requirements.

Hough: Yes, that is quite true. In fact, nowadays, because of the safety of the work force, you are not permitted to taste any of these compounds before they have passed through either a rat or a mouse. In other words, you have to know the LD50 before you can even taste it. All the compounds have been subjected to that initial screen before tasting them. I should add, that when we discovered the sweetness of sucralose, the safety in the work place rules, were not in operation, so we tasted the compounds. In the beginning of the century, of course, Emil Fischer tasted everything that was new. That is how he discovered the sweetness of amino acids and of the L-sugars. I remember that my old supervisor, J.K.N. Jones, always tasted every new product. Of course, it can be a hazardous procedure to go tasting new products and is not to be recommended prior to toxicology screening.

In terms of sucralose, it has undergone thorough and extensive toxicology and pharmacology and it has proved to be safe for human consumption. It has been submitted to the various food authorities, particularly the FDA, for their approval to market it. It is a very expensive procedure, costing millions of dollars to establish the safety of a new compound for use in food and drink.

# THE USE OF HPLC COLOUR ANALYSIS TO INVESTIGATE THE MECHANISM OF RESIN DECOLORIZATION

John C Williams & C L Bhardwaj

Tate & Lyle Research & Technology

## INTRODUCTION

Sugar colours are a mixture of species with different origins. The phenolics in cane or beet give colours by enzymic oxidation and polymerisation. The degradation of sugars also produces colours. Each chemical type will be a mixture of materials with different properties. The mixture of substances is complex and usually only the average properties are measured. It is the averaging of light absorption that gives a smooth visible spectrum. Figure 1 shows how two sugars could have the same ICUMSA colour but differ in their absorbance at other wavelengths because one curve is steeper than the other. This difference would show up as a different hue. A parameter to describe this has been devised, called "N-Value" (Parker 1966). This is related to the average colour molecular weight, and is given by

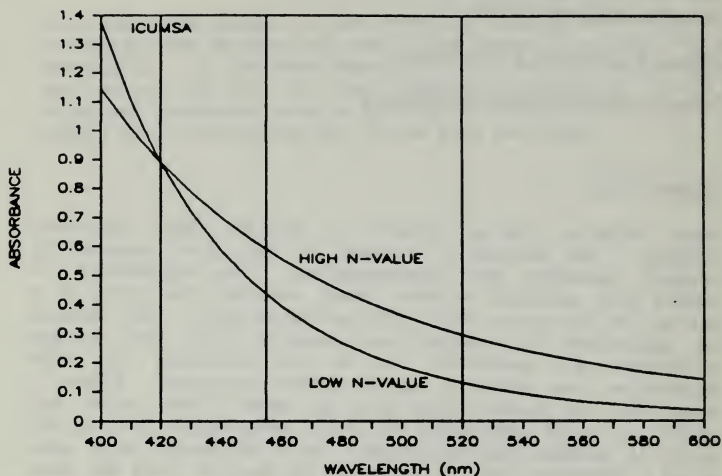
$$N\text{-Value} = 100 \left[ \frac{\text{Absorbance at } 520 \text{ nm}}{\text{Absorbance at } 455 \text{ nm}} \right]^2$$

Measurements of average properties such as the change in absorbance with wavelength or pH (Indicator Value) say very little about the individual colourants that make up the mixtures. Our work has concentrated on two properties of colourant species that are likely to influence their behaviour during processing; molecular weight and charge.

Molecular weight (or size) is an important property because it determines the access of colourants to porous adsorbents. If molecules are larger than the pores, they will be rejected. More subtly, different sized molecules will be rejected by the different sized pores; because no adsorbent has a uniform pore size. Thus there is only a certain range of pores (and hence adsorptive capacity) that is accessible to a molecule of any given size. Size also governs how fast a molecule travels. The larger it is, the slower it moves by diffusion and hence its access to and from adsorbing sites will be affected. The term adsorbing sites is not limited to adsorbents alone but can also cover sites within growing crystals of sucrose or even calcium carbonate or phosphate.

Molecular weight will also be related to the hydrophobicity of a colourant. The more hydrophobic it is, the less affinity it has for water and the more it tends to adhere to surfaces.

Fig 1. SPECTRA OF SUGAR COLOURS



This is important in the action of adsorbents but could also affect the affinity of colours for crystals of sucrose or calcium salts.

The other important colourant property is charge or, more strictly, the number of acidic groups a molecule bears. This is important in ion exchange decolorization. The more charges, the better the colour is removed. It can also be important in the formation of calcium salts in defecation processes. The performance of colour precipitants, such as Talofloc, which react with these acidic groups, is also affected by their number. The combination of molecular weight and charge on a colour molecule (charge density) would also influence the action of precipitants.

It is helpful to visualise the effects of these properties by representing the population of colourants in a sugar liquor in a two dimensional charge-size diagram (Figure 2). It is reasonable to represent colourants as a rough diagonal band. The lower limits of the band would be set by the ability of a molecule to accommodate many charges. The upper limits are intuitively based on the idea of a polymer built up of repeating units; although there is evidence that zero-charged molecules have a wide range of molecular weights. Regions of the charge-size space can be defined that will relate to decolorization processes. In Figure 3 a simplified version is



Fig 2. COLOURANTS  
CHARGE - SIZE DISTRIBUTION

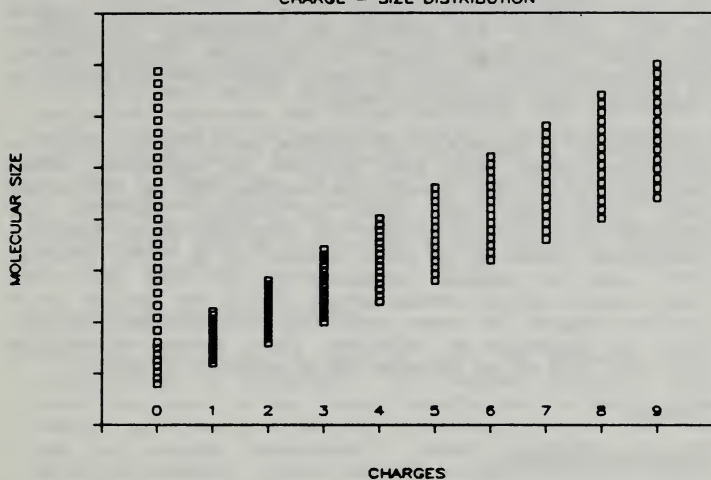
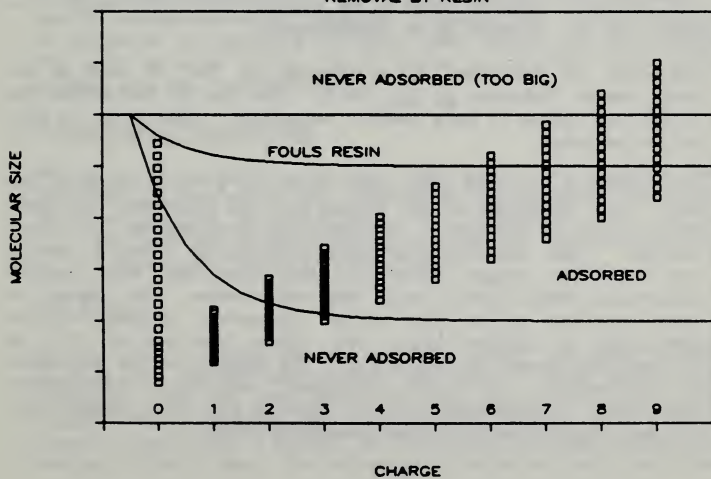


Fig 3. COLOURANTS  
REMOVAL BY RESIN



given of what may be expected in ion exchange decolorization. The largest molecules are rejected. The smallest with lowest charges are not adsorbed because there is insufficient binding to the resin by ion exchange or hydrophobic interaction. Colourants with high charges or molecular weights (highly hydrophobic) will bind strongly and be difficult to regenerate, thus fouling the resin.

Chromatographic methods which separate according to charge or molecular weight will give vertical or horizontal views respectively of this charge size diagram, but no method has been found that will give an overall view of the distribution. Earlier methods of examining these colourant properties (Shore et al 1985, Linecar et al 1978, Williams 1974) used low pressure chromatography and were very slow. High Pressure Liquid Chromatography (HPLC) has enabled us to perform analyses for these properties in well under an hour. Thus the behaviour of colourant types during decolorizing processes such as ion exchange can be examined in detail.

## METHODS

### Colourant Isolation

Before HPLC can be performed, the colouring matters need to be freed from sugars and other impurities which can interfere with separation and detection in chromatography. Spectrophotometric detectors have to be used at 420 nm so that the colour analyses can be related meaningfully to refinery values. Colourants have relatively low extinctions at 420 nm and, as small volumes have to be injected in HPLC, there is a need also for colours to be concentrated.

Isolation is performed by adsorption at low pH onto a non ionic resin (Linecar et al 1978). The low pH deionises the colour and makes it more readily adsorbed. Desorption is with acidic methanol.

This process presents most colourants in a form suitable for HPLC. Two fractions of the colour are not recovered but their amounts can be measured. One fraction (designated "L") leaks through. This contains the most hydrophilic colours, mostly low molecular weight materials; and also the very largest colourants which cannot enter the resin pores. The amount of this fraction is to some extent dependant on the way the isolation is carried out. The other (designated "S") is irreversibly bound to the resin. Its amount can be estimated by difference. It will comprise the most hydrophobic colourants, corresponding to the highest molecular weight that can get into the pores of the isolating resin.



### Charge Distribution

A technique known as "Paired-Ion Chromatography" is used. This can be described as "dynamic ion exchange" where the chromatography is by gradient elution on a reversed phase packing in the presence of a cationic surfactant. Colourants which have more acidic groups per molecule will bind to more surfactant molecules and these complexes will in turn be better retained by the non-polar column. Thus a separation by nett negative charge is obtained. The form of the HPLC trace is shown in Figure 4a. It is too ill-behaved to be integrated by conventional methods, and must be quantified by using Gel Permeation Software to slice the whole chromatogram up and calculate the area of each slice, before assigning peak areas to colour types.

### Molecular Weight Distribution

HPLC Gel Permeation is used to find the molecular weight distributions of colourants. The largest molecules cannot enter the pores of the column packing and so elute first. Smaller molecules are delayed by diffusion within the pores and are eluted progressively later according to their molecular weights. Aqueous elution is used, with the addition of other solvents to minimise adsorption.

The system is calibrated with a series of polyethylene glycol fractions of known molecular weights so that retention times may be related to molecular weight. The molecular weight distribution obtained is divided into arbitrary ranges, to allow easier comparisons of one distribution with another. A smooth chromatogram is obtained (Figure 5a). Quantification is achieved by dividing it into time slices and calculating the area of each slice. Each slice is related to molecular weights via the calibration.

### Chromatogram Presentation

For ease of visualisation and comparison, the chromatograms obtained are converted into histograms representing charge and molecular weight distributions. By convention, the "L" and "S" fractions are placed respectively at the low and high ends of the histograms (Figures 4b and 5b).

### Ion Exchange

A laboratory simulation of a resin plant was set up with acrylic and polystyrene resins in series. The resins were Amberlite IRA 958 and Amberlite IRA 900 C.  $5 \times 10^6$  Bed Volume icu of colour had already been loaded onto the old acrylic resin and  $1 \times 10^6$  BV icu onto the old polystyrene during refinery use. The old acrylic resin had only had brine

Fig 4a PRESSED LIQUOR

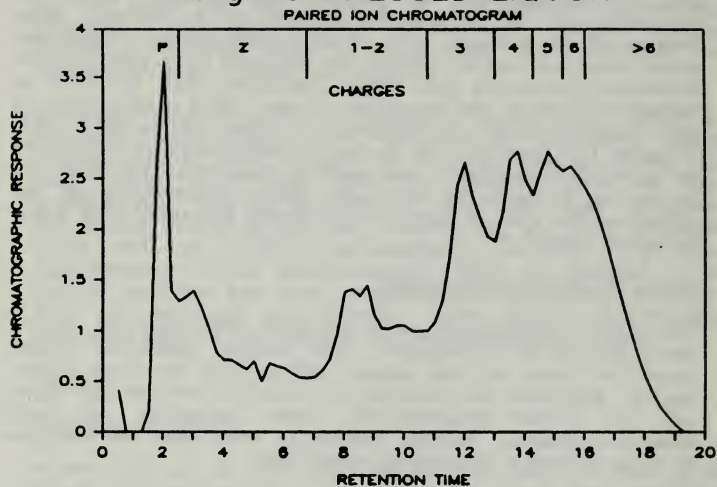


Fig 4b PRESSED LIQUOR

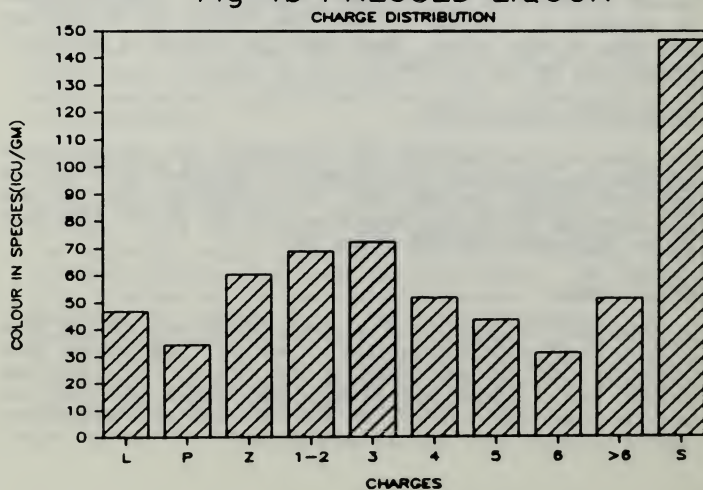


Fig 5a PRESSED LIQUOR  
GEL PERMEATION CHROMATOGRAM

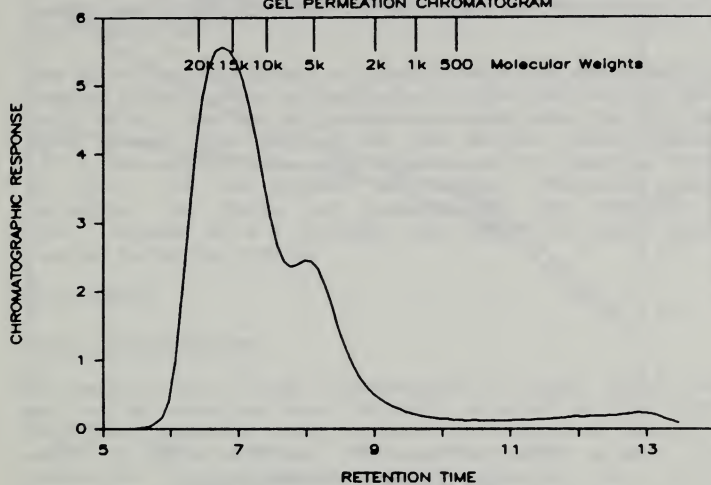


Fig 5b PRESSED LIQUOR  
MOLECULAR WEIGHTS

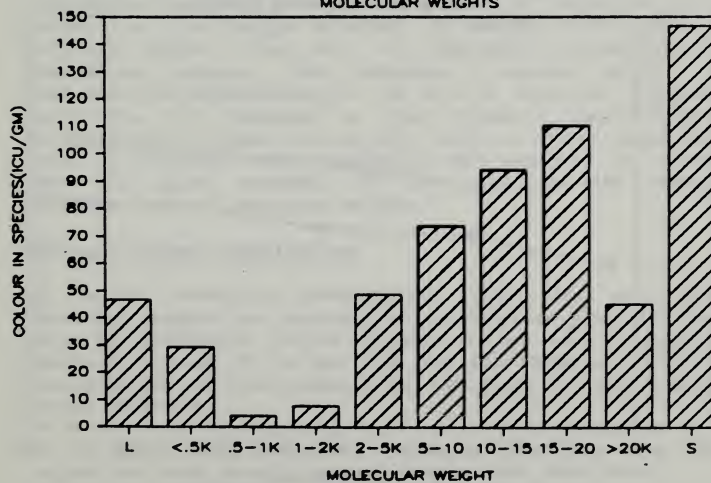


Fig 6a. ACRYLIC RESIN  
DECOLORIZING PRESSED LIQUOR

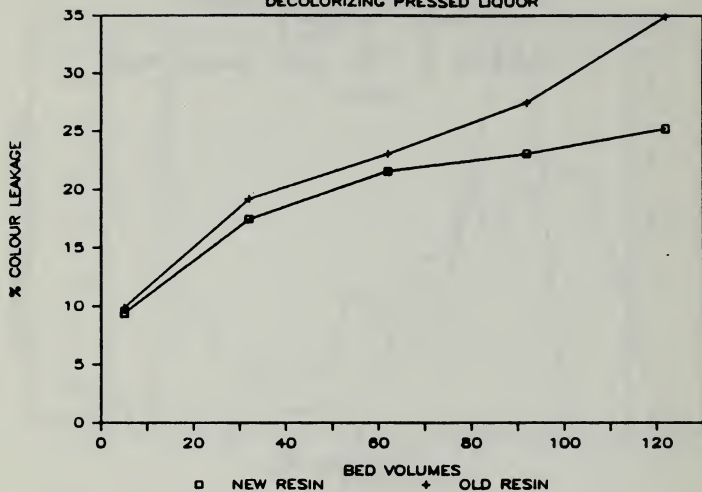
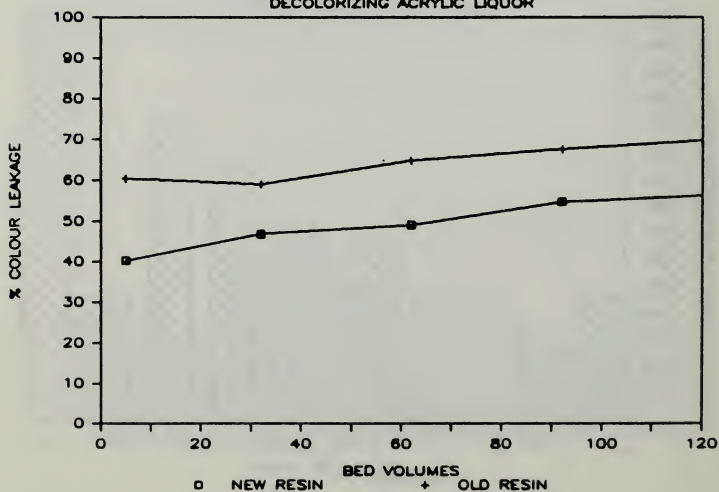


Fig 6b. POLYSTYRENE RESIN  
DECOLORIZING ACRYLIC LIQUOR



regeneration during its service lifetime, so to simulate the effects of a routine alkaline brine regeneration, it was given a prolonged alkaline brine treatment during which considerable colour was eluted. The old styrene resin was regenerated with brine before use.

New and old acrylic resins in parallel were fed with 120 BV of pressed liquor (after carbonatation, colour 606 icu) at 80°C and 3 BV/nr. New and old polystyrene resins were fed with the mixed product from the new and old acrylic resins (colour 139). The acrylic resins were regenerated with alkaline brine at 72°C, and the styrene with brine. Fractions taken throughout the cycles and regenerations were analysed by HPLC. Figures 6a & b represent the amount of total colour leaking during the cycles.

## HPLC RESULTS

### Charge Distributions

The peaks in HPLC charge chromatograms of colour (Figure 4a) are not as sharp as those generally obtained in HPLC work. Each charged species is not a single substance, but contains a range of materials with different molecular weights and polarities. The larger, less polar, materials tend to be better retained on the column and hence emerge later in that charged band. Broad peaks and poor resolution are the result. The charge numbers assigned to the peaks are related back to our earlier work where a DE32 cellulose anion exchanger was used for charge separations (Williams 1974).

The precise nature of the peak designated "P", which is eluted before the Zero charged material, is unknown. It occurs in refinery liquors but not in synthetic glucose/glycine degradation colours. This indicates a natural origin. It must have low hydrophobicity. It is a pH sensitive fraction, indicating the presence of some phenolic functionality. Alternatively, colours with a nett positive charge would come here (if present). Gel Permeation HPLC analyses of colours that are almost entirely "P" show that it can contain colourants of all molecular weights.

### Molecular Weight Distributions

In the gel permeation chromatograms (Figure 5a), all quoted molecular weights are relative to polyethylene glycols. They will be comparable within themselves but not necessarily absolute, because we do not know how the shape of colourant molecules in solution relates to the shape of polyethylene glycol molecules.

It is likely that colourants coming in the "less than 500" region are there because some adsorption does occur even



though it had been minimised by the eluent used. If the separation were entirely according to size, then all material would be eluted before " $V_1$ " (the included volume of the column where the very smallest molecules emerge). The conditions chosen elute more than 95% of the colourants before  $V_1$ . Anything coming beyond this is being further retarded by an adsorptive mechanism. The upper exclusion limit of the column is 20,000 and some material exceeds this. The majority of molecular weights much higher than this will come into the "S" fraction.

## ION EXCHANGE DECOLORIZATION

### Decolorization Mechanism

Figures 7a & b compare the input and output colourant charge and molecular weight distributions for new acrylic resin. Figures 8a & b present this as a % decolorization for each species. The species best removed are the high molecular weights and high charges. The ready removal of highest-charge species indicates that ion exchange is involved, and the efficiency of brine in regeneration supports this. It has been stated (Fries & Walker 1980) that adsorption is the primary mechanism, and the fact that "Zero" charged colourants ("Z" & "P") are removed shows that an adsorptive mechanism is acting. Two factors should favour the removal of highly charged colourants. Multiple binding to the resin is possible if steric factors are favourable. Higher charges are likely to go along with higher molecular weights (with a higher hydrophobicity) so that adsorptive forces may also be important. The overall mechanism could be described as "amplified ionic binding", where the primary attachment is ionic but the additional action of adsorptive forces provides the very strong binding that is observed between resins and colours.

### Colour Leakage

As a resin cycle progresses, more colour appears in the product. The composition of this colour changes and becomes increasingly rich in species with lower charges (Figure 9a). This is reflected in the molecular weight distribution (Figure 9b) where increasing amounts of low molecular weights appear as the cycle progresses. The trends can be expressed as an increasing % leakage of lower charge/lower molecular weight species as the cycle progresses (Figures 10a & b), and can be thought of in terms of displacement of these "lower" species, which have already been adsorbed, by incoming higher charged/molecular weight species which will be more firmly bound.

### Polystyrene Resins

The behaviour of colourant species as a styrene resin cycle progresses is similar to that for an acrylic resin: higher



Fig 7a. NEW ACRYLIC RESIN  
DECOLORIZATION PERFORMANCE

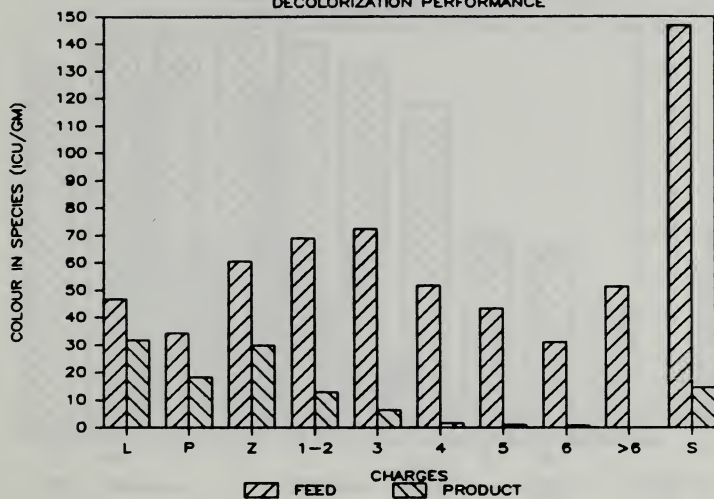


Fig 7b. NEW ACRYLIC RESIN  
DECOLORIZATION PERFORMANCE

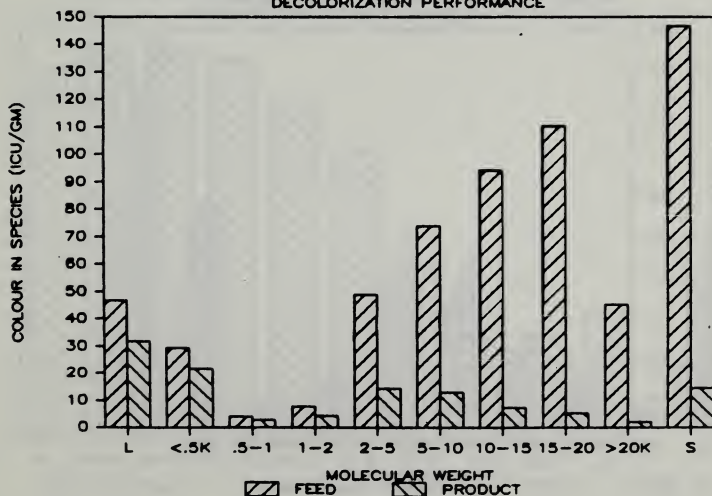


Fig 8a NEW ACRYLIC RESIN

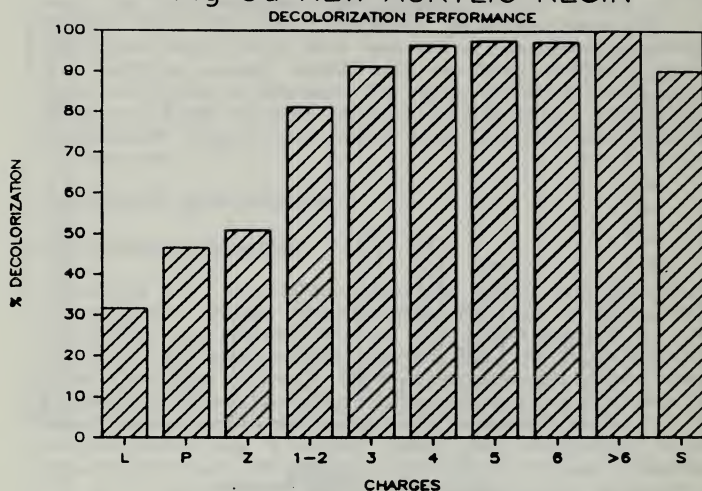


Fig 8b. NEW ACRYLIC RESIN

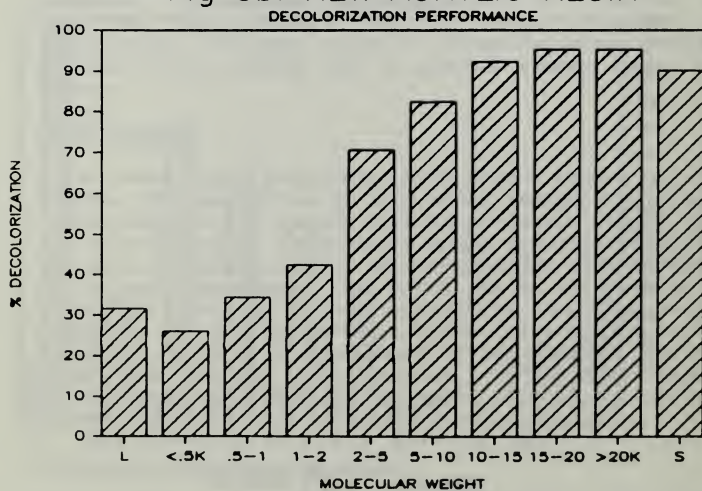


Fig 9a. NEW ACRYLIC RESIN

PRODUCT FRACTIONS FROM PRESSED LIQUOR

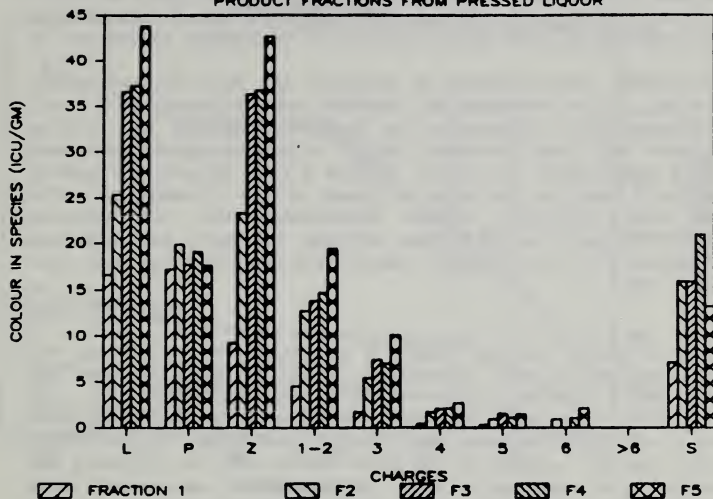


Fig 9b. NEW ACRYLIC RESIN

PRODUCT FRACTIONS FROM PRESSED LIQUOR

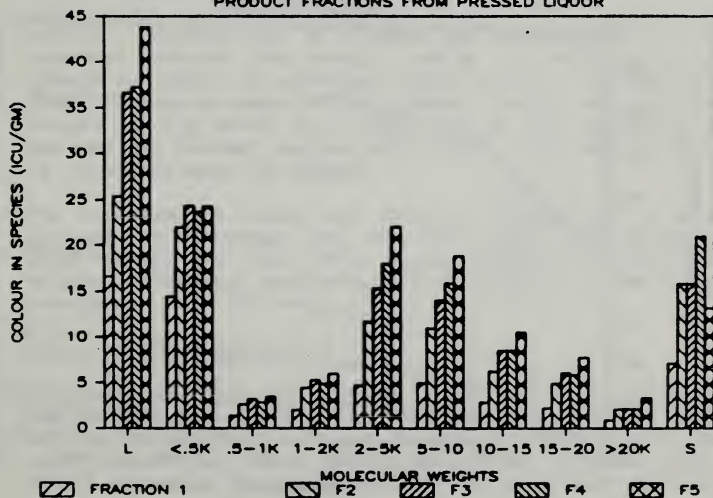


Fig 10a. LEAKAGE OF CHARGED SPECIES

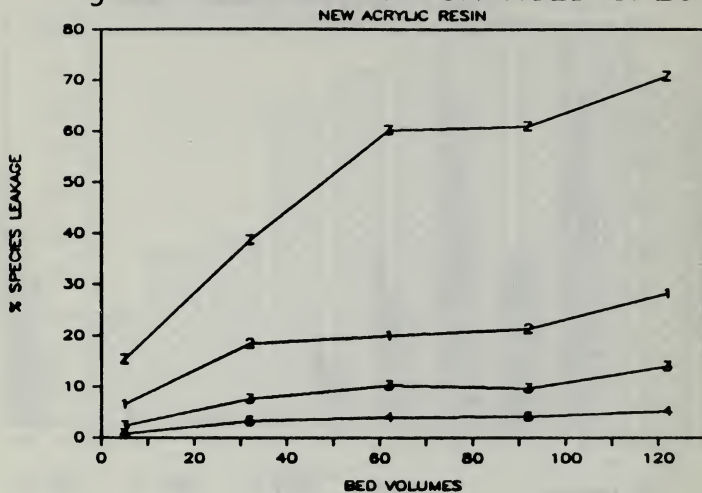
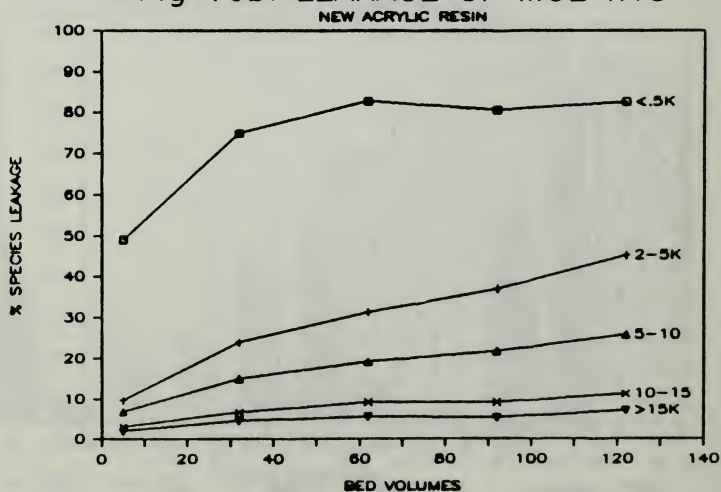


Fig 10b. LEAKAGE OF MOL WTS





charges/molecular weights are best removed and there is a slow increase in leakage of the "lower" colourants as the cycle proceeds (Figures 11a & b). The species with zero charges predominate in the product charge profile, but both types (Z & P) are better removed by the styrene resin than by the acrylic.

The difference from the acrylics is further shown in Figures 12a & b representing the combined performances of old and new acrylic and styrene. Removal of colourants extends further down the charge/molecular weight spectrum when the acrylic product is treated with a styrene resin. A polystyrene resin with its aromatic matrix would be expected to have a higher affinity for the unsaturated colour molecules than the predominantly aliphatic acrylic resin (Fries & Walker 1980). Both adsorptive and amplified ionic binding will be stronger.

### Cycle Profiles

The profile of the change in % colour leakage during a cycle can be simplified by linear regression into a straight line with an intercept and slope. The intercept represents colourants that can never be removed by the resin under the conditions of operation: either they are too large to enter the pores or are too weakly bound to the resin. On the other hand, under the conditions of flow, they have not reached equilibrium with the resin. The slope represents either large molecules no longer able to get into the resin because sites able to accommodate them are already full; or colourants that were initially taken up and weakly bound to the resin and then later displaced by more strongly adsorbed colourants.

This concept can also be applied to each individual colour species. Figures 13a & b show the composition of the intercept colours from acrylic resins in terms of the % leakage of charge or molecular weight species. As expected, there is most leakage of the lowest charges or molecular weights (poorly bound, and eluted even by the low levels of ash that are present) and the very highest ("S", some of which is too big to enter the pores).

The corresponding slopes are shown in Figures 14a & b as increases in % leakage over the whole cycle. As expected, lower slopes are seen with higher charges or molecular weights, reflecting increasingly firm binding of the higher colourants.

### Effect of Resin Age

The cycle profiles for overall colour and for individual species show the effects of resin ageing. In terms of total colour, the intercepts of the new and old acrylics are the same (Figure 6a) Work published earlier (Williams 1984) showed increasing intercepts with age when only brine was used

Fig 11a. NEW STYRENE RESIN  
PRODUCT FRACTIONS FROM ACRYLIC LIQUOR

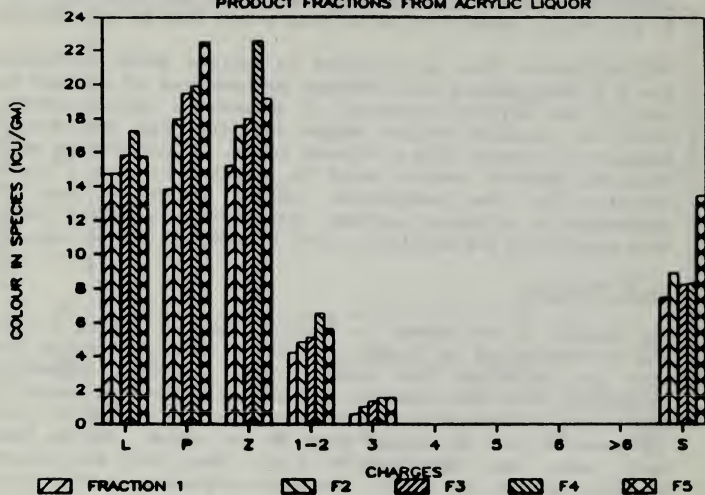


Fig 11a. NEW STYRENE RESIN  
PRODUCT FRACTIONS FROM ACRYLIC LIQUOR

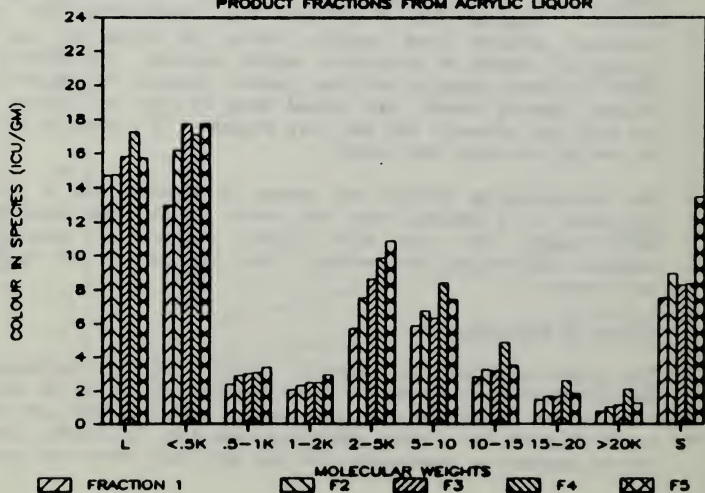




Fig 12a. DECOLORIZATION

CHARGES FROM PRESSED LIQUOR

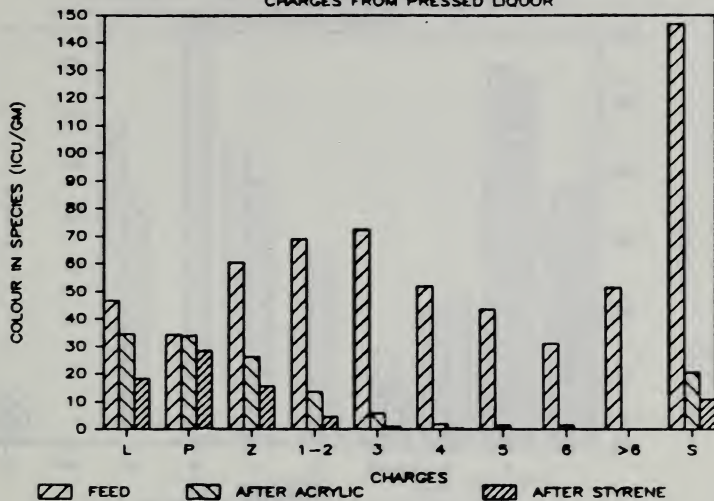


Fig 12b. DECOLORIZATION

MOL WTS FROM PRESSED LIQUOR

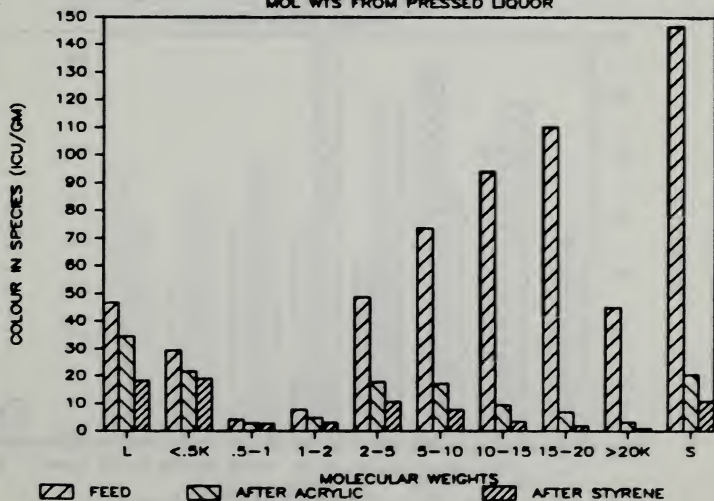


Fig 13a. ACRYLIC RESIN  
SPECIES CYCLE PROFILES

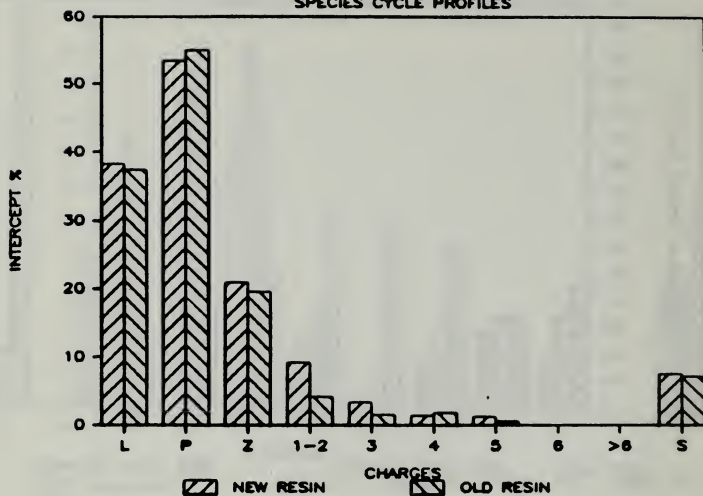


Fig 13b. ACRYLIC RESINS  
SPECIES CYCLE PROFILES

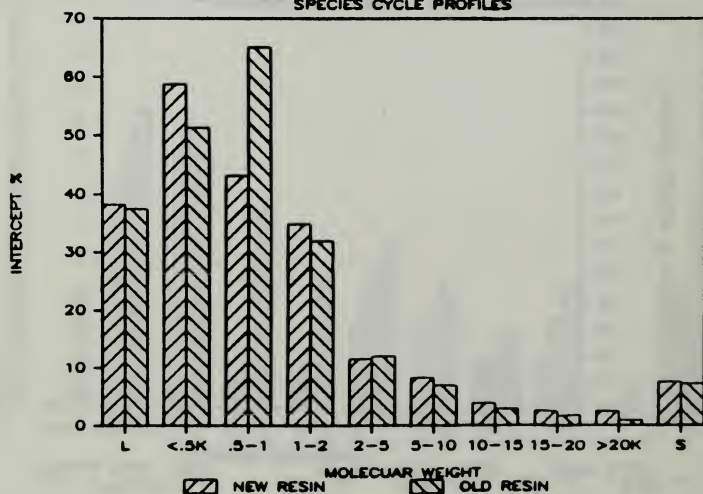


Fig 14a ACRYLIC RESINS  
SPECIES CYCLE PROFILES

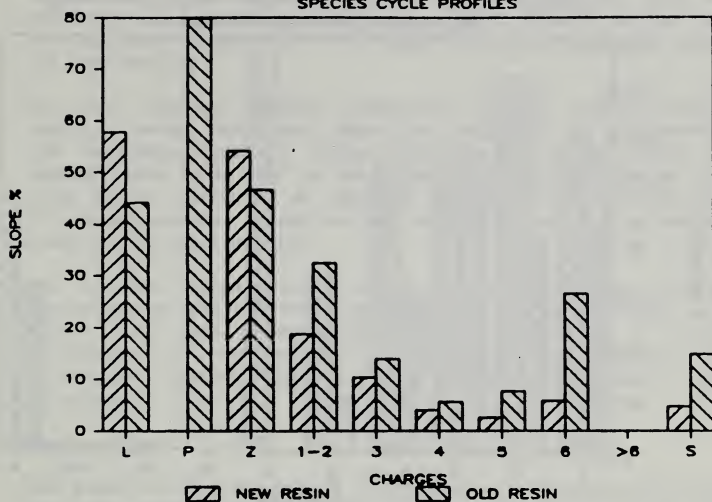


Fig 14b. ACRYLIC RESINS  
SPECIES CYCLE PROFILES

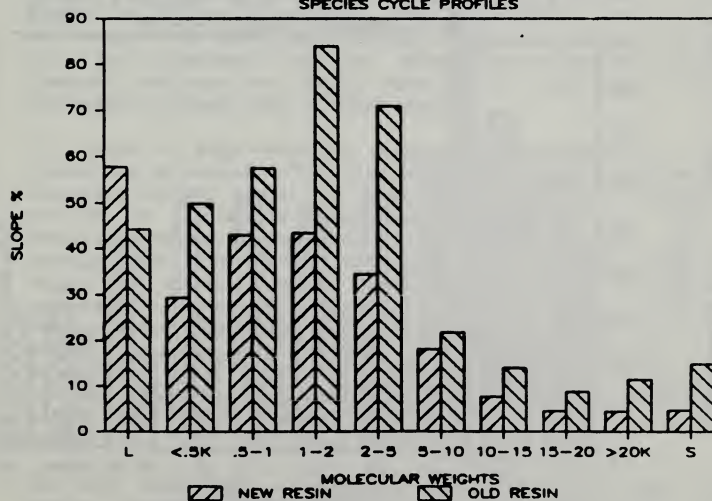


Fig 15a EFFECT OF RESIN AGE  
ON POLYSTYRENE INTERCEPT COLOURS

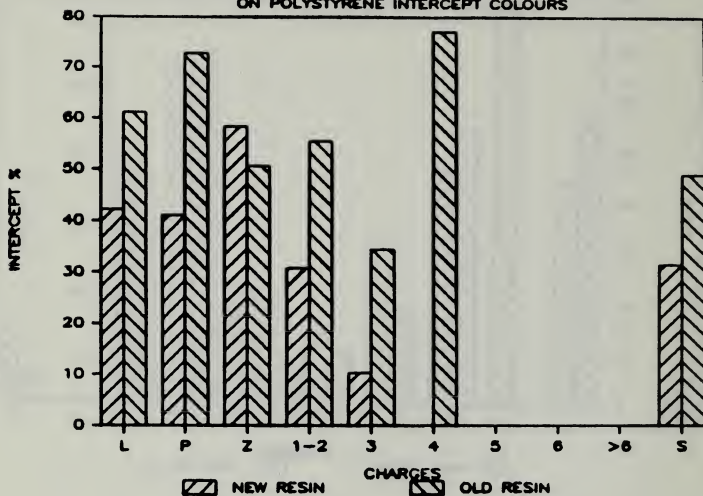
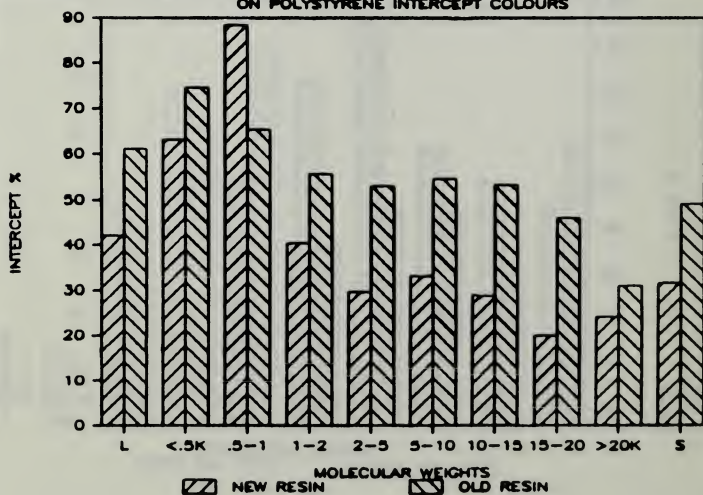


Fig 15b EFFECT OF RESIN AGE  
ON POLYSTYRENE INTERCEPT COLOURS



for resin regeneration. Sites suitable for large molecules had been filled. In this work, the regeneration was effectively with alkaline brine; and the similar form of colourant type profiles for the intercept colours of new and old acrylics (Figure 13a & b) show that the filling of sites is less serious.

For most species, profile slopes are greater for the older resin, showing that less sites are available to accommodate the on-coming load of colour (Figure 14a & b). This is probably due to a loss in ion exchange capacity, although why this should result in an increase of the slope of uncharged "P" is not clear. It may be that regeneration of P is not as complete as other species.

For styrene resins (Figure 15a & b), the colourant species intercepts of the old resin are generally higher than the new. Sites have been irreversibly filled, indicating a lower efficiency of regeneration. This would be expected as polystyrene resins have a higher affinity for colour than acrylics.

### Regeneration

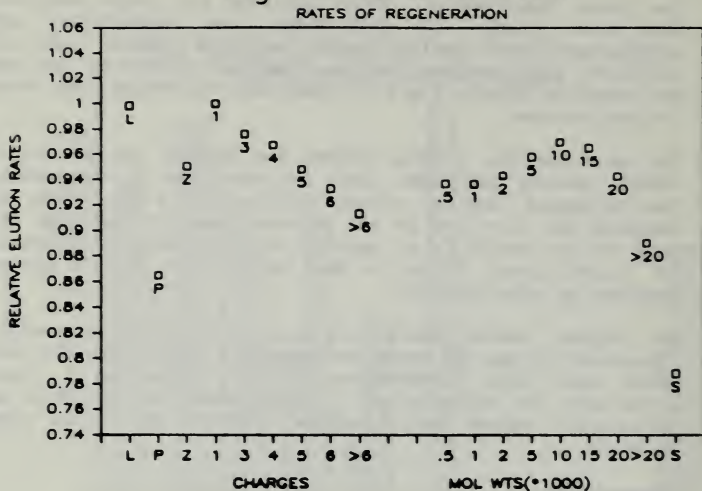
The first 2.5 Bed Volumes (4 fractions) of regenerate from the new acrylic accounted for 89% of the colour. A measure of the rate at which a species is eluted from the resin may be obtained by comparing (on an arbitrary scale) the amount eluted after 3 fractions, with the ultimate elution level. This is shown in Figure 16 for charge and molecular weight species. The sequences may be explained in terms of the properties of the colourants. The higher molecular weights and charges are eluted more slowly. Either the rate at which they are displaced from the adsorption sites is slower (because they are more firmly bound to the resin), or their diffusion is slower (due to their size).

"S", taken as high molecular weight and hydrophobic, is the slowest to be eluted. "P" (zero charge and covering the whole molecular weight range) is also slow. "L" (the adsorbed portion of which is small and hydrophilic) is fast. Decreasing rates in the sequences from 1 to greater than 6 charges, and from 10,000 to greater than 20,000 molecular weight are to be expected. The increase up to 10,000 may represent a shortcoming in our molecular weight analyses whereby some colourants could be assigned a lower molecular weight because of adsorptive retardation during Gel Permeation. This effect may have been emphasised here because the most hydrophobic of the materials in these molecular weight ranges would tend to be concentrated in the acrylic resin.

The same selectivity of regeneration is also shown when the colour remaining on the resin after regeneration is calculated. The consequence of this selectivity is that



Fig 16. NEW ACRYLIC



higher charge/molecular weights will be the species that will foul the resins and shorten their effective life if regeneration is inadequate. Too low a concentration of regenerant will have this effect by failing to displace the most firmly bound colourants. Too little time of contact or too low a temperature would lead to incomplete diffusion of the larger species out of the resin pores.

In the case of the styrene resin, it was observed that the "P" fraction was poorly regenerated, indicating that brine is not a good agent for reversing purely adsorptive binding.

#### CONCLUSIONS

- 1) The highest charged and highest molecular weight colourant species are those most readily adsorbed during ion exchange decolorization. Conversely the lowest or zero charged and lowest molecular weight colourants tend to leak through resins.
- 2) The mechanism of decolorization involves both ionic binding and adsorptive forces. Adsorption may serve to amplify ionic binding but will also act on its own to remove zero-charged colourants.
- 3) The adsorption ability of polystyrene resins relative to acrylic resins extends further down the charge and molecular weight ranges, confirming their greater affinity for colourants.

- 4) As resin cycles proceed, the leakage of lower charged and lower molecular weight materials tends to increase more rapidly than that of higher charge/molecular weight, indicating selective displacement of the lower by the higher.
- 5) Many colourant species leak faster from older resins. This is probably a consequence of less sites being available to adsorb colours.
- 6) A proportion of each colourant species cannot be adsorbed by resins ("intercept colour"). These proportions increase as a polystyrene resin ages indicating fouling due to sites being irreversibly occupied by colourants. This does not show up in an acrylic resin previously regenerated with alkaline brine.
- 7) Regeneration is governed by adsorptive factors i.e. the strong retention of high molecular weight/high charged species, and kinetic factors i.e. the slow diffusion of high molecular weight/high charged species. Thus, correct concentrations and temperature of regenerant are important, as well as sufficient time of contact.

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## DISCUSSION

Question: Thank you very much for an excellent paper. You have added a lot to the understanding of the nature of color molecules. The last diagram you showed points very clearly to the possibility of optimizing regeneration procedures. Would you expand on how you think that might be done.

Williams: It is really a matter of economics, of how much time you can afford to spend on regeneration and how high a temperature you can allow to make diffusion faster and how high a concentration you can get to. Work we did many years ago indicates that about 10% brine, which is generally used, is perhaps an optimum, and you don't get a lot more color removed if you go above that. The other thing, of course, is to add a bit of alkali to the brine, which gives an enhanced regeneration. The mechanism for the action of alkali is not well understood.

Question: It is always enjoyable to listen to your papers because you always present new and interesting approaches on how ion exchange resins work. In the regeneration, as you pointed out, there is an advantage to using sodium hydroxide along with the brine. Is there a practical or economic disadvantage to using it each cycle? You indicated that it is used periodically rather than each cycle.

Williams: There are two disadvantages. One is cost, because sodium hydroxide is expensive, so it is preferable to use it only occasionally. The other disadvantage is that you would be putting some of your ion exchange resin into the hydroxide form, and so enhancing the degradation of the resin.

Question: Are acrylic acid based resins allowed in the United Kingdom?

Williams: These are acrylic based strong anion exchangers and some years ago they received FDA approval, and so they are allowed in the United Kingdom as well.

Clarke, S.P.R.I.: Would you say a little more about the group of colorants you call "P." You said you think they come from the cane plant. As you know, we have done a lot of work at SPRI, too, on color that has been isolated from the cane plant--the phenolic and polyphenolic colorants and on the flavonoid group. Do you think that is what is in "P", because you said it was uniformly removed throughout the resin cycle so I assume a lot of aromatic structure for the absorption. Also, have you isolated this group and looked at it further by HPLC or other techniques?

Williams: The only place from which we have isolated it was a case where we had something that had been through several processes and virtually only "P" was left. So far the only thing we have been able to do with that is to look at the molecular weights. As I mentioned, it extends over the full molecular weight range. Our identification of "P" with a plant origin is actually circumstantial in that we could not find it in the laboratory-induced colorants but we could in the plant colorants. Also, in terms of the indicator value, "P" has a far greater response to pH than the others do.

# ACETIC ACID PRODUCTION IN CANE DIFFUSERS AND THE RESULTANT EFFECT ON VAPOUR PIPE CORROSION IN EVAPORATORS

K.J.SCHÄFFLER

Sugar Milling Research Institute

## INTRODUCTION

Felixton (FX), South Africa's newest and largest cane raw sugar factory was commissioned in 1984. The factory has a capacity of some 3.3 million tons of cane per annum. Two diffuser installations each handle 300 tons of cane per hour. The evaporator station consists of two identical quintuple effects. During 1986 the Institute, in collaboration with Felixton began an intensive study of inversion losses in the evaporator station. At the same time process staff voiced their concern regarding the serious corrosion of vapour and condensate lines, vapour two (V2) was especially affected. Corrosion was severe in condensate pipes and in the steam-chests of vacuum pan calandria. Because of its known aggressiveness in corrosion (Bayers et al.1983) and due to its volatility, acetic acid was determined in V1, V2 and V3 condensates from FX during the 1986 season (Purchase et al.1987). FX results were by far the highest in the Industry with over 80% of the V2 condensate samples containing at least 20 ppm acetic acid. In contrast other mills produced much lower results (Pongola (PG) = 2%, Noodsberg (NB) = 6%, Mount Edgecombe (ME) and Malelane (ML) = 0%). FX's results for last season indicated that acetic acid levels in V1 condensate were generally less than 10 ppm, whilst V2 condensate levels ranged between 30 and 40 ppm and V3 condensate levels were within 20 to 30 ppm. Damage to piping has been considerable and replacement costs have been high. In 1987 the mill was forced to neutralise V2 condensate with a mixture of ammonia and cyclohexylamine. This was, however, only a short term treatment and work was initiated in 1987 to:

- (a) confirm the high acetic acid levels in V2 condensates
- (b) pinpoint where and how acetic acid was being produced
- (c) reduce acetic acid concentrations to acceptable levels.

## EXPERIMENTAL

### Factory Work

V1, V2 and V3 condensates (10 ml) from both sets of evaporators were taken, once per shift, and sealed in pre-labelled sachets. Preservative was not added. The samples were frozen rapidly and stored in a deep freeze. The shift samples were composited to daily samples and these were analysed for acetic acid. Direct analysis of cane extracts (DAC), mixed juice and clear juice samples were taken hourly and composited on either a daily or weekly basis.

## Laboratory Work

Fresh cane was shredded in a Jeffco cutter-grinder. The prepared cane was well mixed and sub-samples (800 g) were sealed in plastic bags and frozen. Water (3500 g) was added to the laboratory diffuser (Lionett 1985) and preheated to 10°C above the predetermined set point, oil at the correct temperature was circulated through the diffuser heating jacket. The cane (800 g) was then dumped into the pot and stirring was commenced. Initially pH was monitored continuously and lime (2.5%) added to bring the pH of the bagasse extract up to set point. Temperature and pH were recorded every 5 minutes, pH was adjusted with a slurry of lime as was necessary. A thermocouple was fitted to the laboratory diffuser and temperature was monitored manually with a digital thermometer. For pH monitoring and control a sample (5 ml) was removed via an ON/OFF valve and placed in a narrow plastic test tube (to avoid evaporation). The pH probe was immediately placed in the tube and the reading obtained from a digital pH meter. Samples (15 ml) were removed every twenty minutes for acetic acid analysis. Total reaction time was 4.5 hours.

## Analysis

Gas Chromatography (GC) was used to determine acetic acid in the samples. Condensates were injected directly, while juice samples were subjected to a vacuum micro-distillation technique prior to GC (Schäffler et al. 1979, Day-Lewis 1985).

## RESULTS

### Condensates

Statistical data for V1, V2 and V3 are shown in Table 1.

Table 1.--Acetic Acid (ppm) in FX Condensates July 87

Condensate Evaporator	V1		V2		V3	
	A	B	A	B	A	B
No. of Samples	25	24	24	25	24	25
Min. ppm	0	1	13	8	3	5
Max. ppm	32	44	129	116	53	62
Mean ppm	10	10	50	44	28	24
SD	7	9	27	26	14	15

Acetic acid levels in V2 condensates for July 1987 are also shown in figure 1. Results from table 1 and figure 1 tend to indicate:

- V1 contained low concentrations of acetic.
- Average levels (50 ppm) in V2 were high, with levels peaking to 110 ppm. Acetic acid concentrations were even higher than those measured in 1986.
- For V3, amounts were intermediate between V1 and V2.



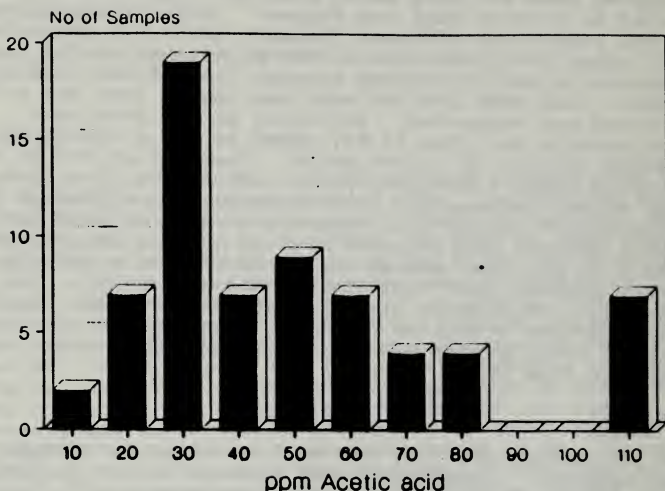


Figure 1.--Acetic Acid in Condensates at FX 1987

#### HPLC

Due to the corrosiveness of acetic acid, the very high levels measured were of obvious concern and the possibility of a co-eluting interferant inflating the results could not be overlooked. HPLC, using both cation exchange and reverse phase columns with ultra-violet detection, was therefore used to cross-check the GC method. HPLC yielded results that were virtually identical to the GC results (Day-Lewis 1987a, Day-Lewis 1987b).

#### Diffusion

Theoretical calculations suggested that invert decomposition in the evaporator could not be the primary cause of the high acetic acid levels. Recently, Ivin (1987) and co-workers in Australia, compared non-sucrose from mills and diffusers as they were concerned that diffusion may extract more undesirable impurities for a given sucrose extraction resulting in higher losses in molasses. The use of lime addition to diffusers was evaluated. Ivin pointed out that the hemi-cellulose fraction of bagasse, although mainly xylan-based, usually carries with it a small amount of acetate esters and in the presence of lime these can

hydrolyse to form calcium acetate. Acetate levels dropped dramatically when the diffusers were not limed. As a direct result of this information FX stopped liming for three weeks. Acetic acid levels in V2 condensates are given in table 2.

Table 2.--Effect of Diffuser Liming on Acetic Acid Levels (ppm) in V2 condensates, FX - 1987

Liming	Mean Acetic	SD	# Samples
ON	50	27	24
OFF	8	5	17
ON	20	10	8

Liming appeared to have a definite effect on acetic acid levels in V2. There was a significant drop when liming was stopped. An increase was again observed when liming was restarted.

### Evaporator Juices

Acetic acid can be measured directly in condensates. To determine this acid in juices a vacuum micro distillation procedure was used (Schäffler 1979). A number of evaporator juices were analysed for acetic acid to determine the effect of diffuser liming on acetate levels. The results are summarised in table 3.

Table 3.--Acetic Acid Levels in Evaporator Juices (ppm on brix). Effect of diffuser liming

Treatment	CJ	1st effect	2nd effect	# Samples
Lime	1610	1770	1650	8
No Lime	420	430	450	6

The levels of acetic acid in evaporator juices show similar trends to the condensate samples, i.e. diffuser liming appears to release acetic acid and raises the levels in evaporator juices. There was also no significant change in acetic acid between CJ and juice exiting the second effect, confirming the theoretical calculation that high concentrations of acetic acid were not connected to monosaccharide breakdown. Approximately 90% of the acetic acid in evaporator juices is present as calcium acetate and is naturally not volatile. Therefore the amount in the vapour is small compared with the amount in juice.



## Factory Trials Of Lime Versus No Lime

The diffusers at FX were run for a period of 19 days without lime addition. MJ acetate levels averaged out at 260 ppm with a scatter of  $\pm 70$  ppm. When liming was re-started the average acetate levels in MJ jumped to 640 ppm with a scatter of  $\pm 150$  ppm. Again liming appeared to have increased acetate levels. In a further attempt to determine the effect of high temperature liming on acetate production, process staff at FX ran the A-diffuser for 1 week at 85°C using their normal liming procedure. The B-diffuser was not limed and it was run at 75°C. The conditions in the two diffusers were then reversed for the following week. Results are included in table 4.

**Table 4.--Effect of Lime and Temperature on Acetate and Lactate Production at FX, 1987 Season (ppm on Bx)**

W/E	Diffuser	Lime	Temp.	Acetic	Lactic
5/12	A	Yes	85°C	960	600
5/12	B	No	75°C	310	610
12/12	A	No	75°C	270	720
12/12	B	Yes	85°C	960	825

The following comments regarding table 4 are appropriate:

- High concentrations of acetate were obtained when either of the diffusers was limed at 85°C.
- When liming ceased, levels dropped to those found in DAC samples (see table 6).
- Temperature and liming appeared to have no effect on lactic acid production. This was expected as even thermophilic bacteria are not active at temperatures greater than 70°C.

## Inter-Factory Comparison Of Acetate Levels In MJ

Weekly MJ samples from 2 mills, 3 cane diffusers and a bagasse diffuser were analysed for acetic acid. The results in Table 5 show:

- Levels of acetate were not significantly different in diffuser and mill juice at MS.
- Although AK has a cane diffuser like FX, AK has comparatively low levels of acetate in MJ, this implies that diffuser liming procedures could be the cause of the significant differences between the two.
- The bagasse diffuser at PG has acetate levels only marginally higher than other factories.
- FX produced the highest acetate levels (1000 ppm) for the period when the factory was liming. This was about 4 to 5 times higher than the other factories. During one week when no lime was added, acetate levels in MJ from FX were similar to the other factories. The evidence to date tends to indicate that the high levels of acetate in MJ at FX are due to the liming procedure.

Table 5.--Comparison of Acetate levels in MJ Samples  
(ppm on Bx)

W/E	PG	FX	AK	MS-D	MS-M	ME
19/9	290	280*	210	260	310	270
26/9	310	N/S	210	270	230	180
10/10	310	900	270	350	310	290
17/10	395	1100	280	300	250	260
MEAN	330	-	240	295	275	250

\* Diffuser not limed N/S no samples (floods)

PG - Bagasse Diffuser (uses lime)

FX - Cane Diffuser (lime added at eight points)

AK - Cane Diffuser (lime added at two points)

MS-D - Cane Diffuser (lime added at one point)

MS-M - Milling tandem ME - Milling tandem

acetate in MJ at FX are due to the liming procedure.

#### DAC Samples

Acetic acid can also be produced by bacteria at temperatures lower than those normally used in diffusers (Macrory et al. 1984). In order to acquire more information on background levels of acetic acid prior to bagasse hydrolysis, DAC samples were tested for both acetic and lactic acids over a five day period. Results from table 6 indicate that:

- Background levels of acetic acid entering the factory lie between 200 and 300 ppm on brix.
- Due to the high diffuser temperatures used at FX any increase in acetic acid over the background level is probably an indication of acetyl hydrolysis rather than micro-biological breakdown of sucrose.
- Lactic acid levels, a sensitive indicator of bacterial loss of sucrose (McMaster et al. 1974), were always higher and more variable than acetic acid concentrations.

Table 6.--Organic Acids in DAC Samples, FX w/e 29/11/87  
(ppm on Bx)

Date	Bx	Acetic	Lactic
25/11	5.19	230	390
26/11	5.13	210	430
27/11	4.95	260	360
28/11	4.80	310	310
29/11	4.68	280	830
Mean	4.95	260	460
SD		140	±210

## Changes In Acetic Acid Across Clarification

Most of the data presented in the preceding sections have pointed to the hydrolysis of acetyl esters in the diffuser as being the main cause of high acetic acid levels in evaporator condensates. Preliminary examination of acetic/brix ratios between MJ and clarified juice (CJ) indicated additional formation of acetic acid. Weekly samples of MJ and CJ from 2 diffusers and a mill were examined and results reported in table 7.

Table 7.--Changes in acetic acid during clarification  
(ppm on brix)

Mill	MJ	CJ	% Increase
FX	400	540	35
	250	360	44
	430	580	35
	340	440	29
	300	440	47
AK	150	220	47
	160	270	69
	180	270	50
	150	250	67
DL	270	440	63
	260	400	54
	210	300	54

The results clearly indicate a substantial increase (36-60%) in acetic acid during clarification. MJ inevitably contains some bagasse, which either gets through the diffuser bed or through the mill's MJ screens, leading to further ester hydrolysis as reaction times are long (1½ hours) and temperatures and pH's are also high. Bagacillo is also added to the clarifier muds to assist in filtration and again conditions in the mud-mixer favour acetic acid formation. Although these observations were not investigated further, the effects of ester hydrolysis during clarification, on corrosion in evaporators and on molasses exhaustion will receive attention at the SMRI during 1989.

## Laboratory Work On The Effect Of pH And Temperature On The Rate Of Acetate Release From Bagasse

Laboratory diffusers were used to examine the hydrolysis of acetyl groups in bagasse. Average scatter for temperature and pH was  $\pm 1.0^{\circ}\text{C}$  and  $\pm 0.07$  pH units respectively. The level of precision is probably acceptable as set-point differences between the various runs were much greater. The concentration of available acetyl groups was reported by Trickett (1982) to be 4.5% on dry weight of bagasse. The results of kinetic



experiments to determine the order of the hydrolysis reaction and the rate constants under different pH and temperature conditions are shown in table 8.

Table 8.--Summary of Kinetic Data for Acetyl Hydrolysis:  
Effect of pH and Temperature

No.	pH	Temp.	Intercept	k (/min)	Corr. Coeff.	Half-Life (Hours)
6	5.83	86.3	7.2356	-3.89E-05	0.9921	297
7	6.41	85.5	7.2370	-1.23E-04	0.9953	94
8	7.35	85.8	7.2493	-6.38E-04	0.9970	18
9	7.50	72.8	7.2460	-3.67E-04	0.9976	31
10	7.50	82.1	7.2653	-9.61E-04	0.9937	12
11	7.50	90.2	7.3015	-1.97E-03	0.9953	6

It is obvious from Table 8 that the hydrolysis of bagasse acetyl groups is indeed first order, i.e. under defined conditions of temperature and pH the rate of hydrolysis is only dependent on the acetyl concentration in bagasse. The concentration of available acetyl groups in bagasse was calculated experimentally using the Guggenheim procedure (Bamford et al.1969), the experimental result (4.9% on dry weight of bagasse (Schaffler 1988)) was similar to the figure quoted by Trickett (1982). It must be remembered that all pH values quoted in this study were measured at operating temperatures, pH values at room temperature will be marginally higher (Schaffler 1987). The combined effect of these 2 variables on the rate constant, k was determined by subjecting the data to multi-linear regression analysis. This procedure yielded the following equation:

$$\log k = 4.2453 + 0.9446 \times \text{pH} - 5086/(273 + T) \dots (1)$$

A correlation coefficient of 0,983 was obtained. This single equation describing the effect of pH and temperature on k can be used to determine the production of acetic acid in diffusers under different conditions. For example during the 1987 season (Schaffler et al. 1988) it was observed that when the diffusers were not limed acetic acid production dropped almost 4-fold. It can be shown from equation (1) that an increase of 0.6 pH units is necessary to quadruple acetate production. An increase in temperature almost always results in an increase in the reaction rate. Again from equation (1) it can be shown that a 10°C rise will increase the reaction rate 2.5 times. The combined effect of pH and temperature is shown in figure 2. To illustrate the use of this graph, two different diffuser conditions are examined. Diffuser A is held at 80°C and is not limed (pH at 80°C is 5.0). From figure 2 it can be seen that 0.06% acetic acid is produced for a three hour bagasse retention. For diffuser B the temperature is 90°C, pH is held at 6.5, 4.2% acetic acid is produced in the same reaction time. Of course the choice of an optimum pH and temperature regime is further complicated by the effect these two variables have on

corrosion, inversion and invert decomposition rates in the diffuser. If the diffusers are not limed corrosion damage to the diffuser will eventually result in considerable replacement costs, especially as cane delays result in average pH values for MJ of 5.0-5.1. Inversion losses at these pH levels (coupled with high temperature (85°C) and long retentions (1-2 hours)) will also be significant. The current liming procedure on the other hand cannot continue, neutralisation of condensates to avoid vapour pipe and vacuum pan corrosion is costing FX \$80 000 per annum.

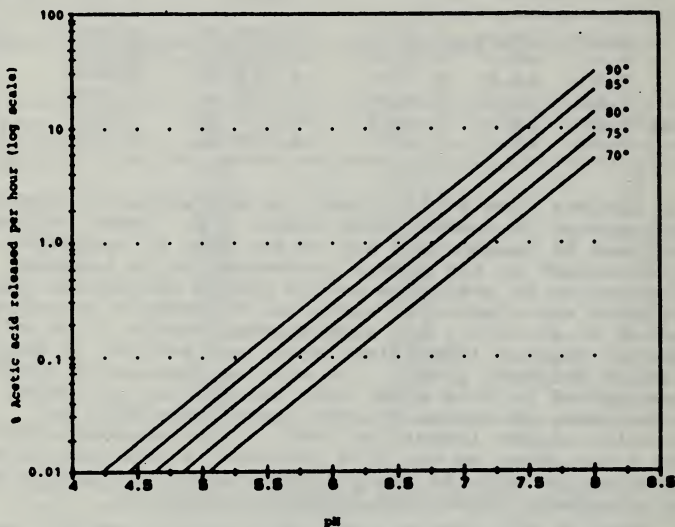


Figure 2.--Hydrolysis of acetyl-ester groups in bagasse at different temperatures and pH's.

#### FUTURE WORK

The investigation into acetic acid production from the hemi-cellulose fraction of bagasse will continue:

- FX use a 12 baumé lime solution to control pH in the diffusers, a second, more dilute (3-4 baumé) liming tank has been installed in an attempt to reduce pockets of high pH.
- Different liming procedures will be attempted.
- As temperature is also important, this will be reduced to 80°C in the first three stages with the back-end being held at 72°C. Lactic acid levels will be monitored to check on bacterial activity.
- Additional ester hydrolysis in the clarifiers and in the mud filters needs to be investigated.

- e) A model incorporating retention times, pH and temperature needs to be developed to optimise variables in terms of inversion and acetic acid formation.

## SUMMARY

FX mill has been plagued by serious vapour and condensate pipe corrosion. The problem arises as a direct result of high acetic acid levels in vapours, particularly in vapour two. The high levels of acetic acid are a direct result of the liming procedures used in the Felixton diffusers. When liming stopped acetate levels dropped to acceptable levels. Additional ester hydrolysis appears to be occurring in the clarifiers and mud-filters. Kinetic work in the laboratory has resulted in a simple model describing the combined effect of pH and temperature on acetic acid formation. The model will hopefully assist process staff in arriving at a compromised set of conditions to keep sucrose inversion and diffuser corrosion to a minimum, whilst reducing acetate levels in juice which in turn will decrease vapour and condensate pipe corrosion in the evaporators.

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## DISCUSSION

Question: You mentioned that when you have acetic acid and you are at the distillation stage, it is usually neutralized with ammonia or cyclohexylamine. I can see that ammonia might be good because it is cheap. Cyclohexylamine sounds a bit expensive and less volatile, but still volatile. Why not use a non-volatile cation? Why not put the lime in after you have removed the bagasse? The cation stays there and is not lost like ammonia is. It would be much more effective then.

Second, your investigation on the effects of liming have concentrated on the acetic acid that is produced. It would be very good if you took into account the hemicellulose that is extracted by lime, much more so than is extracted by water. I think you should be looking at the polysaccharides coming into the juice with liming as well as just acetic acid coming into the juice.

Schaffler: A mixture of ammonia and cyclohexylamine is used as a corrosion inhibitor for vapours and steam lines. Because ammonia is volatile it will neutralise a considerable amount of acetic acid in the vapours. Cyclohexylamine is not volatile under conditions in vapour 2 (b.p. 132-138°C), and will therefore coat the surface of the piping reducing or preventing corrosion. The mixture is available commercially and is known as Mogul SL-395, it has been approved by the Food & Safety inspection service of the US Department of Agriculture.

With respect to your second question, on solubilising more polysaccharide with lime juice, this would indeed be the case. However the problem of corrosion at FX was so serious that we concentrated on acetate production rather than increased polysaccharide levels.

Question: Even in the most neutral solution, you will get some hydrolysis of acetyl esters, so you will always get some acetic acid. What about my idea of adding lime after you remove bagasse?

Schaffler: There are two reasons for adding lime to a diffuser. The pH of MJ can be as low as 4.8 to 5.2, and lime is added to preserve the diffuser which is fabricated out of mild steel. In addition, sucrose inversion can occur at these low pH-values especially if temperatures are high and throughput rates are low.

Question: As you know, in the beet sugar industry, we destroy all our invert sugar completely and we make acetic acid and we have the same range of acetic acid concentration in our process as you do. However, we have no acetic acid in our condensates because the pH of the thin juice is much higher than in your case, and I think this is the problem.

Schaffler: In beet juices the levels of invert are extremely low, and it is then possible to destroy them in the clarifier at high pH. The levels of invert in cane juices are much higher; destruction of significant quantities of invert would result in extremely dark juices; molasses exhaustion will also be affected. During evaporation the pH of the ingoing clarified juice must not be above 7.0-7.1; otherwise colour and acid formation from invert would result.

## HIGH MOLECULAR WEIGHT COLOR IN REFINERIES

M.A. Godshall, M.A. Clarke, C.D. Dooley and E.J. Roberts

Sugar Processing Research, Inc., New Orleans, LA 70179

### INTRODUCTION

The concern about color in sugar -- its formation, removal and prevention -- is shared by the sugar industry worldwide and has been a unifying theme almost from the beginning of sugar refining. Many papers have appeared over the years about color, no fewer now than in the past, attesting to the complex nature of the colorant(s) and the continuing challenge that color presents to the refiner and manufacturer.

Since sugar color does not represent discrete, easily characterized, components, it is convenient to divide it into various categories, according to origin -- sugarcane plant or factory-derived; according to basic chemical type -- enzymatic, melanoidin, caramel, polyphenolic, alkaline degradation; according to pH sensitivity; according to the degree of charge; or the range of molecular weight. These categories all give valuable information about the nature of various colorants and provide clues to control and removal.

The high molecular weight (HMW) colorant is of particular interest because it has a tendency to increase during processing, to be more difficult to remove, and to be preferentially occluded in the crystal (Gross, 1967; Smith, 1967; Parker and Williams, 1969; Tu, et al., 1974, 1977; Kennedy and Smith, 1976; Shore, et al., 1984; Mantovani et al., 1986; Godshall et al., 1987; Godshall and Clarke, 1988). Tu defined colorants of molecular weight 5000 (daltons) and above to be high molecular weight, and examined their behavior in raw sugar manufacture. Roberts and Godshall (1979) examined HMW colorants in refining by dialysis using molecular weight cut-off values of 3000, 8000, and 12,000 daltons. In recent studies, HMW colorant above 12,000 daltons has been studied (Godshall et al., 1987; Godshall and Clarke, 1988) and above 20,000 daltons (Clarke et al., 1987; Godshall et al., 1987). These studies have shown that a significant proportion of colorant in crystals is of molecular weight greater than 20,000 daltons. The proportion increases from raw sugar (36-40%) to washed raw sugar (43-83%) to refined sugar (60-100%).

In the study reported here, dialysis (molecular weight cut-off of 12,000 daltons) and gel permeation chromatography (GPC) were used to observe



changes in HMW colorants in refineries, in white boiling systems, and in the remelt station. The technique also provided information on polysaccharides in process.

## MATERIALS AND METHODS

Samples were obtained from several refineries of S.P.R.I. sponsor companies in several areas, and included process samples from the remelt station and the white boiling side. Color was determined by the ICUMSA method (Schneider, 1979); phenolics and total polysaccharides were determined using standard S.P.R.I. methods (Roberts, 1982; Clarke, et al., 1984). Color greater than 20,000 daltons molecular weight was determined by membrane ultrafiltration.

Dialysis. To obtain a sugar-free extract of high molecular weight components, sample containing the equivalent of 100 g solids was dialyzed in regenerated cellulose bags with a molecular weight cut-off of 12,000 daltons. After dialysis, samples were freeze-dried, giving a dry, stable, sugar-free material.

Gel permeation chromatography. The HMW colorants were separated on a 2.6 x 70 cm column packed with Sephacryl S-500 (dextran crosslinked with acrylamide) to a height of 43 cm. Sample was introduced via a pump to the bottom of the column and separated by upward flow, at 2.75 ml/min, using deionized water as the eluting solvent. Components in the sample were detected by both ultraviolet (UV) light, at 214 nm, which was sensitive to colorants, and by refractive index (RI), sensitive to carbohydrates and salts. Peak areas were integrated by the in-house Hewlett Packard Laboratory Automation System.

Laboratory-scale decolorizing tests. Two high color raw sugars (ICU color = 5886 and 7894) from the Caribbean and South America were decolorized in jacketed 1.1 x 60 cm columns, containing a volume of 20 ml settled sorbent, heated to 70° C. Fresh sorbent was used for each separation. Solutions containing 100 g sugar at 25 brix were passed through the columns at a flow rate of approximately 10-12 ml/min. After color determinations, the treated solutions of the Caribbean sugar were dialyzed for examination of the HMW colorants. Sorbents used included IRA-958 anion exchange resin in the chloride form (product of Rohm & Haas), Cane-Cal Carbon (product of Calgon Corp.), XAD-2 and XAD-4 (products of Rohm & Haas), and virgin bone char (provided by a sponsoring refinery).

Sorbents were prepared as follows: Char and carbon were prewashed with hot (90° C water) and decanted until free of fines. IRA-958 was washed with water to remove all residual color and fines and then loaded with 10% NaCl. It was washed with water until the eluent was negative for chloride ions. XAD-2 and XAD-4 were washed with methanol to remove fines and residual color, then rinsed with water to remove methanol.

## RESULTS AND DISCUSSION

### The High Molecular Weight Colorants

Before beginning a discussion of the behavior of the various HMW colorants in the refinery, it is helpful to describe them. Figure 1 shows examples of typical raw sugar HMW colorant profiles, detected by UV. While each sugar is different, some generalizations are possible. There are basically 3 major colorant areas, which are diagrammed in Figure 2. Within these three peak areas, there can be a lot of variation.

The first peak, which, for the purpose of this discussion, is called the "very high molecular weight colorant", or VHMW colorant, is the first peak to elute from the column, and has the highest molecular weight, greater than 1,000,000 daltons. It is often a doublet, as shown in Figure 1(a,b,d). Occasionally, it is a single peak, as shown in Figure 1(c). This peak is associated with both polysaccharide and a hazy turbidity, and has a light yellow color. The turbidity will pass through an 8  $\mu$  filter and also goes through the GPC column, but will not pass through a 0.45  $\mu$  filter.

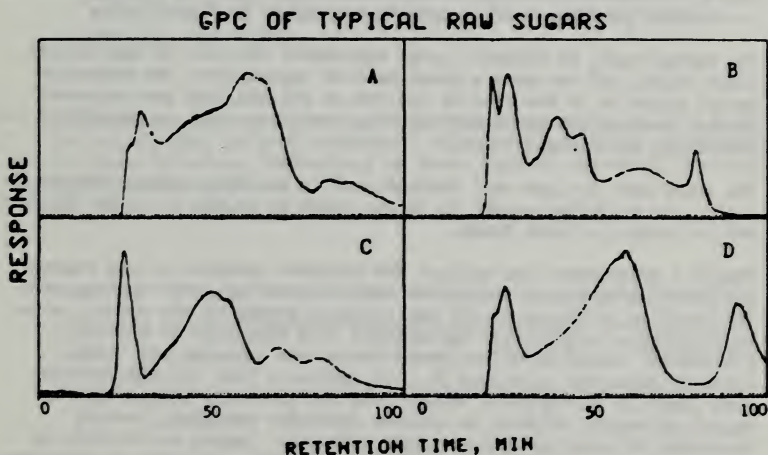


Figure 1.—Gel permeation chromatography of typical high molecular weight colorants in raw sugars. The first peak on the left is the highest molecular weight and the first to exit the column. All peaks are >12,000 daltons.



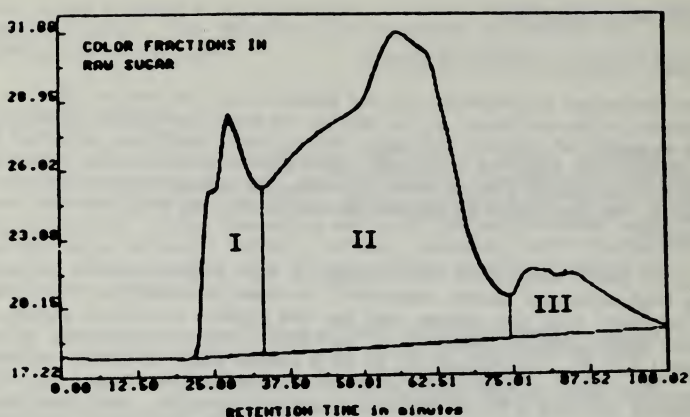


Figure 2.--The three basic high molecular weight colorant areas in typical raw sugars. (See also Table 1.)

Peak I is sometimes, though rarely, missing in some raw sugars, as shown in colorant profiles of less-typical raw sugars in Figure 3(a).

The second peak, or colorant area, represents the bulk of the visible brown color, and can show a great deal of variability. The molecular weight range is in the area of 100,000 to 500,000, and can represent several overlapping components as shown in Figure 1(b) or consist of one peak only, as in Figure 1(d).

The third peak is less well defined, and is sometimes absent (Figure 3-a,b). Its molecular weight is in the range of 20,000 to 50,000 daltons, and the color is light brown.

Figure 3 shows some less typical H<sub>2</sub>O colorant profiles of raw sugars. The profiles in Figure 3(a,b) are seen in some very high quality raws. The profiles in Figure 3(c,d) may indicate damage to the sugars, as we can duplicate this pattern by heat and acid treatment of a normal dialyzate. There is an increase in both V<sub>2</sub>H<sub>2</sub>O colorant and "low" colorant (Peak III in Figure 2). It indicates that the mid-colorant peak (Peak II in Figure 2) breaks down and partially polymerizes to higher molecular weights, as well as insoluble material, leaving fragments of lower molecular weight colorant. Hence, an increase is seen in the higher and lower ends of the profile, with the concomitant loss of the mid-range colorant. Binkley (1970) discussed the formation of the browning polymer with heat, and Feather and Nelson (1984) showed the concomitant formation of both insoluble material and soluble high molecular weight polymers in model browning systems.

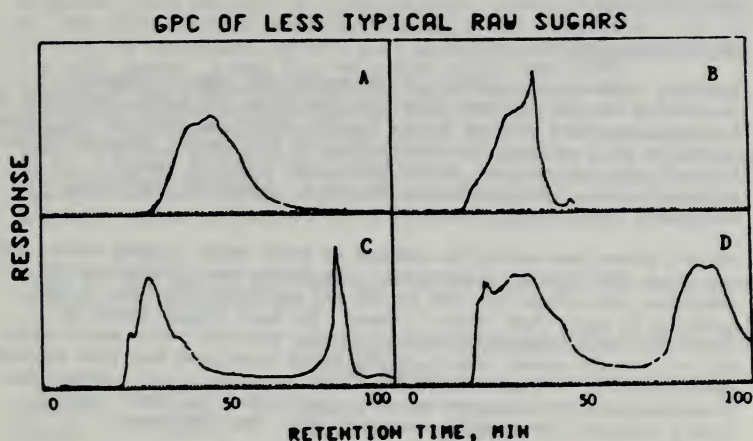


Figure 3.—Gel permeation chromatography of less typical high molecular weight colorants in raw sugars. Raw sugars in (a) and (b) lack very high molecular weight Peak I and sugars in (c) and (d) have increased amounts of lower range colorant Peak III than normal.

The notation used in Figure 2, for broad colorant ranges in a typical raw sugar, will be used throughout this paper. Some of the particulars of these colorants, determined by tests done on recovered fractions, are listed in Table 1.

Table 1. Characteristics of HMW colorants in a typical raw sugar

Peak	MW, daltons	Color	Relative Area %	Relative Color %	Relative Phenolics %
I	>1,000,000	Yellow	16.3	10.7	11.6
II	100,000 to 500,000	Brown	74.6	77.4	77.7
III	20,000 to 50,000	Brown	9.1	11.9	10.7

## REFINERY STUDIES

### The Refinery Process Results

Refining consists of a series of processes that ideally removes increasing amounts of impurities from the input raw sugar, producing a high quality white refined sugar. In this study, we examined the changes occurring in a refinery in some components that are of interest to the study of HMW components: ICUMSA color, HMW colorant peaks, polysaccharides, GPC polysaccharide peaks, phenolics, and amino nitrogen.

Table 2 shows the cumulative changes in total color (ICUMSA color) and the three HMW colorant components. The latter were determined by comparing peak areas obtained by GPC and relating this back to the quantity of sugar from which it came. The melt liquor showed an increase in all components except colorant Peak II. This melt liquor did not contain remelt sugar, so the extra input load was from recycled sweetwaters, a fact evident in the large increase in polysaccharides. Clarification provided some recovery, but washed raw sugar removal levels were not consistently exceeded until char treatment.

This recycling of color and polysaccharides in sweetwaters, and the additional load on refinery process, has been described in recent papers (Clarke and Blanco, 1986; James, et al., 1986).

Table 2. The fate of high molecular weight colorants in a refinery, determined by GPC

Process Sample	Percentage of colorant removed, relative to the raw				
	Total ICUMSA Color	ICUMSA Color Removed	1/2HMW Colorant Removed (Peak I)	1/2Mid Colorant Removed (Peak II)	1/2Low-range Colorant Removed (Peak III)
Raw	2225	--	--	--	--
Washed raw	1054	52.6	74.2	59.7	53.3
Melt liquor	1444	35.1 (+17.5)*	0 (+14.3)	61.1	38.0 (+15.3)
Clar. liq.	1369	38.5	71.4	56.2	57.8
1st liq/char	506	77.3	67.8	74.9	96.7
1st liq/carb	120	94.7	80.3	93.4	97.3
1st strike	6	99.7	98.9	99.4	97.9
2nd strike	17	99.2	86.1	99.4	97.3

\*Percent change from washed raw, representing an increase in component.

A similar trend was seen for polysaccharide removal, detailed in Table 3. The polysaccharide peaks are detected by the RI detector, and in this series of samples, as with most sugar process samples, consisted of two major peaks: A very high molecular weight peak (VHMW polysaccharide), associated with the Peak I colorant, which includes indigenous sugarcane polysaccharide (ISP) and dextran, if any is present. The second peak is about 30,000 daltons and represents Robert's glucan (Roberts, et al., 1986), now known to be a phytoglycogen (Roberts, et al., 1988). It can also include small, soluble degraded starch or dextran. Small amounts of other polysaccharides are also present. (Refer to Figure 4 for a typical polysaccharide profile).

Table 3. The fate of high molecular weight polysaccharide components in a refinery, determined by GPC.

Process Sample	Total Poly-sacch. (ppm)	Percentage of component removed, relative to the raw sugar		
		Total Polysac. Removed	% VHMW Polysac. Removed	% Robert's Glucan Removed
Raw	1043	—	—	—
Washed raw	946	9.3	44.1	60.4
Melt liquor	1263	0 (+21.1)*	0 (+13.8)	0 (+31.4)
Clar. liq.	978	6.2	9.7	61.8
1st liq/char	687	34.1	12.3	87.0
1st liq/carb	570	45.3	38.5	87.3
1st strike	350	66.4	75.4	88.1
2nd strike	596	42.9	49.2	88.4

\*Percent change from washed raw, representing an increase in component.

As with the colorant, it is also possible to note significant increases in polysaccharide in the melt liquor, resulting from recycling. The glucan was evidently removed fairly well by clarification and char, but washed raw sugar removal levels of VHMW polysaccharide were not achieved again until crystallization of the first strike sugar. Polysaccharide levels can increase rapidly in subsequent crystallizations, as noted in the increases that occurred in total polysaccharide and VHMW polysaccharide in the second strike sugar. (Refer also to Table 5 for incremental changes with each process.)

The determination of phenolics and amino nitrogen is of interest because these components can give an indication of the composition of colorant in a raw sugar (Clarke et al, 1986, 1987). The behavior of these components is shown in Table 4. The decrease in amino nitrogen in the melt liquor and/or the increase in clarified liquor could be an artifact of analysis since the absolute value of the differences between processes are small. Between the washed raw and the first strike sugar,



only 38% additional amino nitrogen was removed. The poor removal of amino nitrogen throughout process has been observed before (Clarke et al 1986, and unpublished results).

Table 4. Fate of phenolics and amino nitrogen in a refinery

Process	Phenolics (ppm)	%Phenolics Removed	Amino N (ppm)	%Amino N Removed
Raw	417	—	58	—
Washed raw	290	30.5	36	37.9
Melt liquor	305	26.9 (+3.6)	28	51.7
Clar. liq.	286	31.4	32	44.8 (+6.9)
1st liq/char	154	63.1	28	51.7
1st liq/carb	63	84.9	24	58.6
1st strike	44	89.4	14	75.9
2nd strike	48	88.5	12	79.3

Another way to look at the information in Tables 2, 3 and 4, is to consider the incremental changes, as a percentage, in components that occur with each process step, rather than the cumulative changes from the input raw, which are shown in the previous tables. Table 5 shows the incremental changes for each of the components, allowing a ready comparison of the efficiency of each process for removing each component.

Table 5. Incremental percent removal of components from raw sugar by each process in a refinery

Process	Amino N	Phenolics	ICUMSA Color	Colorant Peak I	Colorant Peak II	Colorant Peak III
Raw	0	0	0	0	0	0
Washed raw	37.9	30.5	52.6	74.2	59.7	53.3
Melt liquor	13.8	+ 3.6	+17.5	+88.6	1.4	+15.3
Clar. liq.	+ 6.9	4.5	3.4	85.9	+ 4.9	19.7
1st liq/char	6.9	31.7	38.8	+ 3.6	18.6	39.1
1st liq/carb	6.9	21.8	17.3	12.5	18.5	0.5
1st strike	12.3	4.5	5.1	18.6	6.0	0.6
2nd strike	3.4	+ 1.0	+ 0.5	+12.8	0	+ 0.6

Table 5., cont'd.

Incremental percent removal of components from raw sugar by each process in a refinery

Process	Total Polysac.	VHMW Polysac.	Robert's Glucan
Raw	0	0	0
Washed raw	9.3	44.1	60.4
Melt liquor	+30.4	+57.8	+91.8
Clar. liq.	27.3	23.5	93.2
1st liq/char	27.9	2.6	25.3
1st liq/carb	11.2	26.2	0.3
1st strike	21.1	36.7	0.7
2nd strike	+23.5	+26.1	0.3

### The White Boiling End

Samples were obtained from a refinery with four white boilings, which included B-syrup, 3rd sugar, C-syrup and 4th sugar. This set of samples contained HMW colorant Peaks I, II and III, as well as both of the major polysaccharide peaks. Tables 6, 7 and 8 show the analyses obtained on these sugars. While excellent removal of most constituents was obtained, amino nitrogen, polysaccharides and VHMW colorant Peak I were relatively less well removed from the mother liquor than the other components examined. Obviously, if the starting raw had been available, the removal factors from the raw would have been much lower.

Table 6. Color in white end samples

Sample	ICUMSA Color	%Color Removed	Phenolics (ppm)	%Phenolics Removed	Amino N (ppm)	%Amino N Removed
B-syrup	1412	—	377	—	50	—
3rd sugar	38	97.3	23	93.9	20	60.0
C-syrup	3149	—	747	—	60	—
4th sugar	45	98.6	31	95.9	14	76.7



Table 7. High molecular weight color in white end samples

Sample	Color >20,000	%Color >20,000	%Color >20,000 Removed	%Colorant Peak I Removed	%Colorant Peak II Removed	%Colorant Peak III Removed
B-syrup	391	27.7	—	—	—	—
3rd sugar	24	63.6	93.9	77.6	93.5	61.1
C-syrup	161	5.1	—	—	—	—
4th sugar	17	37.5	89.4	80.6	91.0	94.4

Table 8. Polysaccharides in white end samples

Sample	Total Polysac. (ppm)	%Polysac. Removed	%VHMW Polysac. Removed	%Glucan Removed
B-syrup	1917	—	—	—
3rd sugar	527	72.5	76.8	29.2
C-syrup	4025	—	—	—
4th sugar	829	79.4	82.6	83.8

Figure 4 compares the colorant in the C-syrup to that in the 4th sugar. Peak I was the most difficult of the HMW colorants to remove, only 80.6% being removed by crystallization, compared to 91% and 94.4% respectively for removal of colorant Peaks II and III.

Previous studies have shown that polysaccharides are difficult to remove in process (Roberts, et al., 1978; Godshall, et al., 1987) and are preferentially occluded in the crystal (VanHook, 1983; Godshall, 1988). Significant quantities of both polysaccharides, shown in Figure 5 for the third syrup, have gone into the crystal.

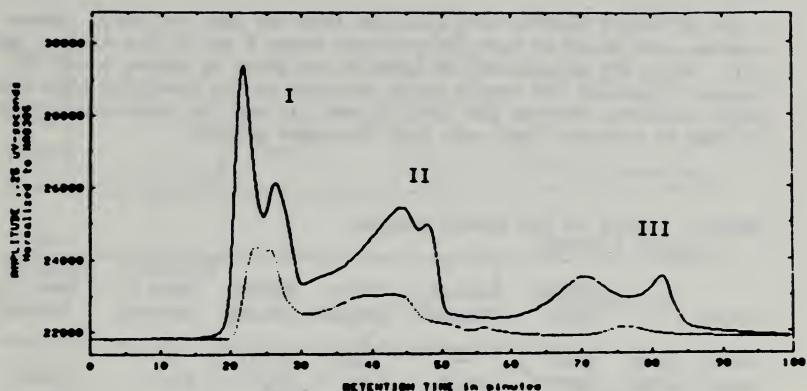


Figure 4.--High molecular weight colorant in a fourth sugar boiling.  
Top tracing: C-syrup (1.25 g). Bottom tracing: Fourth sugar (6.14 g)

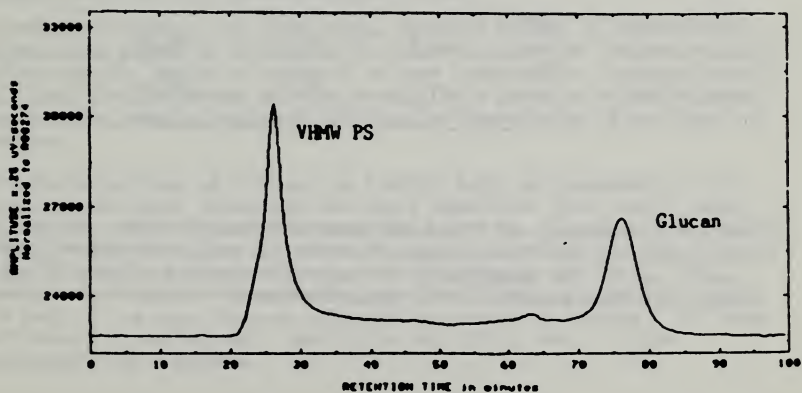


Figure 5.--Polysaccharide in third sugar (16.6 g).

### The Remelt Station

A set of remelt samples were obtained from the same refinery. These samples were found to have GPC colorant Peaks I and II but minimal peak III. Color and polysaccharide results are shown in Tables 9 and 10. Figure 6 compares the remelt syrup colorant to the resulting high remelt sugar colorant, showing the lack of Peak III and the large concentration of Peak II colorant that went into the sugar crystal.

Table 9. Color in the remelt station

Sample	ICUMSA Color	%Color Removed	Color >20,000	%Color >20,000	%Colorant Peak I Removed	%Colorant Peak II Removed
Remelt syrup	4746	—	522	11.0	—	—
Remelt sugar	3497	26.3	1364	39.0	83.3	80.8
Cryst. syrup	5121	—	1521	29.7	—	—
Cryst. sugar	2790	45.5	1121	40.2	60.8	79.6

Table 10. Polysaccharides in the remelt station

Sample	Total Polysac.	%Polysac. Removed	%VHM Polysac. Removed	%Glucan Removed
Remelt syrup	1617	—	—	—
Remelt sugar	547	66.2	80.1	65.1
Cryst. syrup	1594	—	—	—
Cryst. sugar	205	87.1	80.3	63.6

Unlike the refinery and white end samples discussed above, the remelt color data for the GPC colorants do not match well with over-all color removal data. One possibility for this discrepancy is that much of the total color was present in the syrup film as color <20,000 daltons.

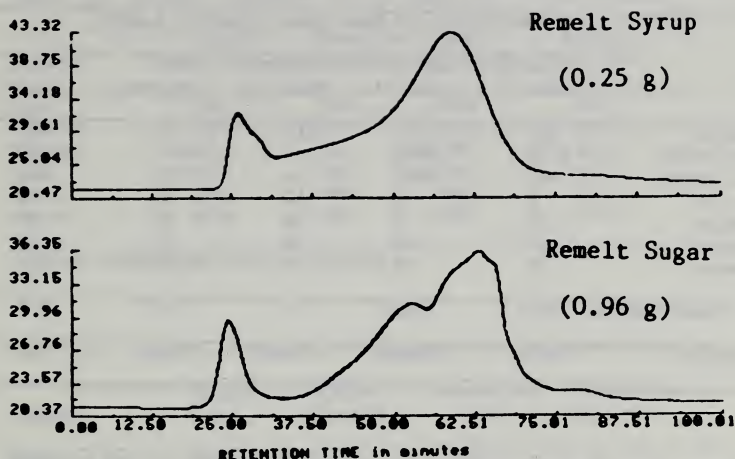


Figure 6.--The high molecular weight colorants in remelt. Top tracing: Remelt syrup (0.25 g solids); bottom tracing: Remelt sugar (0.96 g solids) Note the lack of colorant Peak III in these samples.

#### Types of Color Within Raw and Refined Crystal

A comparison made of the peak areas, obtained by GPC, of the various crystalline sugars in this study (the two remelts, the raw, and the four white sugars), yields an estimate of each component in the sugar. Table 11 lists the peak areas (as area counts) on a per gram of solids basis, so that the numbers represent quantitative comparisons of one sugar to another.

The concentrations of colorant in a white sugar are dependent on the starting raw sugar along with the extra input load from remelt sugars and dilution sweetwaters. The weighted ratios of colored sugars (raw and remelt) to white sugars, in Table 11, provide a measure of the relative ease of removal of each GPC component: The greater the ratio, the greater the removal. Fortunately, the most highly colored peak, which is Peak II, is also the most effectively removed in process. The ease of removal is as follows: Peak II >> Peak III > Peak I > VHMW polysaccharide = glucan.

Table 11. Concentrations of individual GPC components in crystalline sugars.

Sugar	Concentration of Peak (Area counts/g sugar)				
	Colorant Peak I	Colorant Peak II	Colorant Peak III	VHMW Polysac.	Glucan
Raw sugar	446	1391	618	195	293
Remelt sugar	1172	8902	—	611	137
Cryst. sugar	943	9002	—	573	283
1st sugar	5	8	13	12	35
2nd sugar	62	9	16	99	34
3rd sugar	28	25	4	47	39
4th sugar	79	95	8	155	100
Raw + Remelt:White	20:1	188:1	—	5:1	5:1
Raw :White	10:1	40:1	60:1	2:1	6:1

Once again, the results show that the highest molecular weight material, Peak I, stays through process and goes into the white sugar and is highly concentrated in remelt sugars, adding to the process load.

#### LABORATORY STUDIES

##### Laboratory Scale Tests with Absorbents: Effect on HMW Components

It was of interest to determine the affinity of absorbents for the various GPC peaks. The series of processes in a refinery is designed to maximize color removal in steps so as not to put too much burden on any one step. Less is known about the removal of polysaccharides in process, but studies show that they are less easily removed, and so the ideal is to have very low polysaccharide in the input.

In the tests reported here, two high color raw sugars were treated with conventional refinery absorbents, bone char, carbon and anion exchange resin, as well as the two XAD resins, of interest for research purposes. Table 12 gives the results of absorbent treatments on raw sugar No. 1 (from the Caribbean), and Tables 13 and 14 give the results for raw sugar No. 2 (from South America).



Table 12. Sorbent treatment of raw sugar No. 1 (Caribbean)

Sorbent	ICUMSA Color	Phenolics (ppm)	Color >12,000*	%Color >12,000
None	5886	894	2005	34.1
IRA-958	955	577	165	17.3
Carbon	2227	211	1289	57.9
Char	3533	443	1736	49.1
XAD-2	4481	684	1941	43.3
XAD-4	5324	749	2000	37.6

\* Color determined after dialysis.

Table 13. Sorbent treatment of raw sugar No. 2 (South America)

Sorbent	ICUMSA Color	%Color Removed	Phenolics (ppm)	%Phenolics Removed
None	7894	--	807	--
IRA-958	712	91.0	271	66.4
Carbon	4570	42.1	367	54.5
Char	4034	48.9	598	25.9
XAD-2	6173	21.8	674	16.5
XAD-4	6648	15.8	731	9.4

Table 14. Effect of sorbent treatment on high molecular weight colorant in raw sugar No. 2 (South America)

Sorbent	Color >20,000*	%Color >20,000	% Color >20,000 Removed
None	1707	21.6	--
IRA-958	81	11.4	95.3
Carbon	1070	23.4	37.3
Char	1564	38.8	8.4
XAD-2	(3270)	53.0	**
XAD-4	1071	16.1	37.3

\* Color determined with membrane filters.

\*\* Color increase is anomalous; may have formed on column



Dialysis and GPC were performed on the high molecular weight components of raw sugar No.1, with the results obtained in Table 16. Under the conditions of the test, the IRA resin had the best performance, with nearly 92% removal of color above 12,000 daltons, but no effect on removal of the Peak I colorant. Figure 7 compares the IRA-treated colorant to the original raw sugar, showing the dramatic removal of the Peak II colorant, while having no effect on the Peak I colorant. Char was the only one that removed any amount of Peak I, 68 and 39% respectively. About 78% of the color >12,000 daltons was in Peak II, which was best removed by IRA.

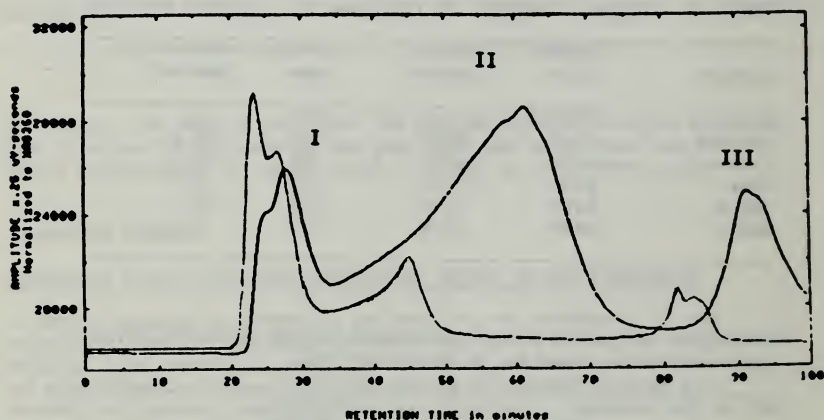


Figure 7.--Effect of treatment of a raw sugar with IRA-958 anion exchange resin in the chloride form on HMW colorants. Top tracing: Raw sugar; bottom tracing: Raw sugar after treatment with resin.

Table 16. Effect of sorbent treatment on individual high molecular weight components in Sugar No. 1 (Caribbean)

Sorbent	Percentage of Component Removed by Sorbent					
	Peak I	Peak II	Peak III	Robert's Glucan	Color >12,000	Total Color
IRA-958 (C1)	0	87.3	68.7	86.0	91.8	83.8
Carbon	0	25.2	93.2	100	35.7	62.2
Char	68.4	5.0	74.7	23.9	13.4	40.0
XAD-4	0	0	0	91.7	0.2	9.5
XAD-2	0	0	51.1	0	3.2	23.8

#### SUMMARY

Gel permeation chromatography, using the new support, Sephacryl S-500, has permitted the separation and differentiation of the very high molecular weight colorants and polysaccharides in sugars and process samples. Individual components were followed through the process, the effect of recycling through remelt and sweetwaters was observed, and their inclusion in the final white sugar product was noted. A ratio has been developed which gives an estimate of the probability that a colorant or polysaccharide will go through process and crystallize with the white sugar.

Three major colorant areas were characterized, comprising broad molecular weight ranges of greater than 1,000,000 daltons (Peak I), 100,000 to 500,000 daltons (Peak II) and 20,000 to 50,000 (Peak III). These colorants comprise, on average, 30-50% of the color in a raw sugar. Within these ranges, there is some variation, but they have distinct characteristics and different behaviors. Peak I is of particular interest and concern because it is associated with polysaccharide and fine turbidity, and both have a tendency to be occluded in the refined sugar. Peak II comprises the bulk of the visible high molecular weight color and tends to be effectively eliminated in the refining process, but builds up quickly in subsequent crystallizations.

An area of possible future research is to develop a quick-screening technique for raw and washed raw sugars to determine the make-up of the high molecular weight colorants -- a profile of the colorants. The technique is slow at present because of the required dialysis step, and ways to eliminate or speed up this step can be explored.

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## DISCUSSION

Question: I have two questions. First, based on the experience that we have had with gel permeation chromatography of polysaccharides and of polymeric tannins, are you sure that your chromatography profiles are reproducible? By that I mean, if you take a single batch of freeze-dried dialyzate and run it in the normal manner, then take the same sample, put it in water and boil it for 5 min and thirdly, if you want to go that far, try it in molar urea, which breaks hydrogen bonds. We have often found that those very high molecular weight peaks, the excluded peaks, are associated molecules rather than covalently bonded.

The second question is regarding Peak II. I think you said that if you treat peak II with acid, it disappears and you get more Peak I and Peak III. Is that correct?

Godshall: That is correct, but I want to emphasize that these peaks do not represent single entities. By hot acid treatment of Peak II, we have degraded it to produce both more highly polymerized material that shows up in Peak I and some lower molecular weight material that shows up in Peak III. In addition, with this treatment, we produce a lot of insoluble, very dark, almost black residue.

Question: An alternative explanation could be that Peak II contains polymeric carbohydrate and flavonoid material bonded, behaving intermediately. You separate them on a polysaccharide column. Although you have smaller polymeric flavonoids, they now interact with the color material and behave like bigger molecules. I don't know if that is the answer.

Godshall: That may well be part of the answer. What we are calling Peak II is detected by UV at 214 nm; it does not give a significant R.I. response, indicating only minor polysaccharide content. The response is above baseline, but is not a peak. It is also visible to the eye as brown colorant. The polysaccharide is associated more with Peak I and to a lesser degree with Peak III.

In response to your first comment, you may be right that the very large colorants represent associated molecules. However, these are what are found throughout the refinery and going into the crystal, and so we are interested to look at them in their aggregated states.

Dickert, Rohm and Haas: In the past, we have talked about these XAD resins. I guess I have been a little bit negligent not to suggest others, simply because we have not sought FDA clearance for them. But your work has shown the effect of different resins, and I wonder if from an analytical point of view in trying to understand what is happening, maybe it would be appropriate to include an acrylic XAD to see how it might work compared to the polystyrene types or others.

Godshall: That would be worth looking into.



Question: We have been looking at colorant profiles as well, and we find it very difficult to relate the peaks we get at 254 nm back to color at 420 nm. We get marvelous looking peaks but we can't seem to understand the actual relationship of the peaks to the color. This is a big problem for us.

Godshall: The 214 nm detection is much more sensitive than 420 nm is. In order to relate the UV peaks to the visible color, we recover the various peaks detected at 214 nm and concentrate them down and read their absorbance at 420 nm. Both the relative peak area and 420 absorbance of the peaks correlate very well to total color. Table 1 in the paper shows this relationship.

## LABORATORY STUDIES ON PSEUDOMONAS PAUCIMOBILIS

Brian Dewar and Vicky Wang

Redpath Sugars

### INTRODUCTION

It is virtually impossible to eliminate bacterial contamination in the sugar refining process. Bacteria enters at various stages in the process: with raw materials, through handling the product, during transportation or even from the air. However, rigorous sanitation coupled with constant monitoring can keep bacteria well within accepted limits.

Redpath rarely has a problem with such contamination. In fact, our vigorous program of product monitoring and heavy emphasis on sanitation throughout all phases of the refining process have resulted in product quality well above industry standards.

Included in our monitoring efforts is a microbiological testing program with schedules for all areas of the refinery. Each schedule is designed to monitor general or specific areas of the process stream, including handling systems and final products. As a result, we are immediately aware of any microbiological changes in the system, and are able to react quickly to potential problems.

It was through routine testing of our liquid sucrose loads and storage tanks in the beginning of 1987 that standard plate count analyses revealed a high level of yellow pigmented bacterial colonies (Fig 1). This isolate was significantly different than the low levels of non-pigmented *Bacillus* species colonies normally found. While our total count was within standards, the presence of this new organism was increasing plate count levels close to the 200 CFU/10 gm sucrose criterion, a level well above our operating norm.

As a result, we initiated an investigation. We intensified our monitoring efforts throughout the refinery as part of a program to:

- 0 Determine the identity of the bacterium
- 0 Trace its portal of entry
- 0 Institute measures to eliminate or control it.

Because the contaminant was unknown to us, we began pasteurizing our end product to ensure its quality before it left the refinery. This gave us time to pursue our investigations.

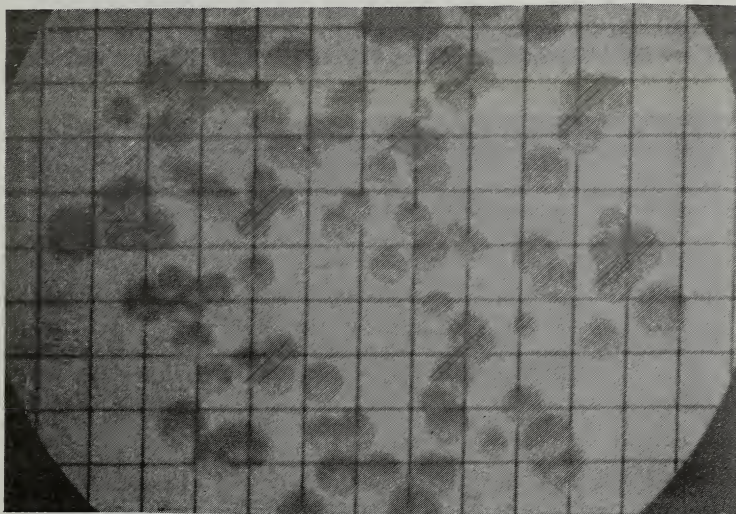


Figure 1. Colony Characteristics of Redpath Isolate on Nutrient Agar. Mag. approximately 3X.

#### IDENTIFICATION OF THE ISOLATE

In order to identify the bacterium, a series of screening tests were performed on a pure culture of the isolate, followed by more extensive testing of the organism's biochemical reactions.

The screening procedure, a modification of one described by Cowan and Steel (1974), included:

- 0 a Gram's stain procedure;

- 0 observation of cell shape and cell arrangement (Fig 2).;
- 0 examination for the production of endospores;
- 0 an oxidase test;
- 0 a catalase test;
- 0 determination of motility
- 0 and a test to determine the ability of the isolate to oxidize or ferment glucose (OF-glucose test).

In addition, tests for growth on MacConkey Agar (Difco) and flagellar anatomy (Mayfield and Innis, 1977) (Fig 3).

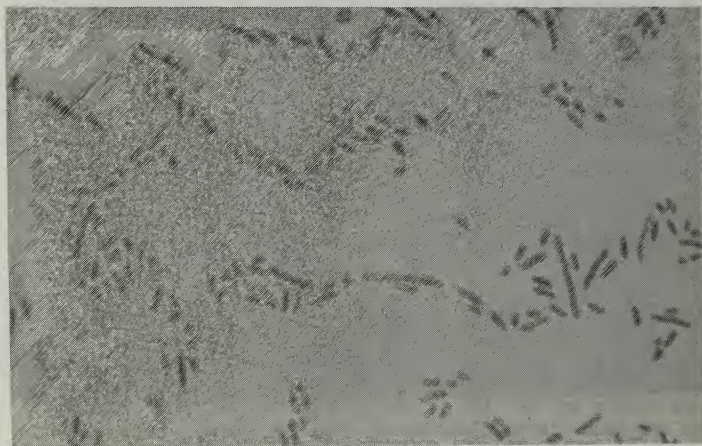


Figure 2. Grams Stain Showing Cell Shape and Cell Arrangement of Redpath Isolate. Mag. 8330.

were performed. We made several modifications to these tests to screen the isolate. To observe cell shape, cell arrangement, motility and spores, a wet mount preparation of cells taken from Nutrient Gelatin Yeast Extract Agar plus 0.005 gm per litre  $MnSO_4$  was examined microscopically, using phase contrast at 1250X magnification. Catalase activity was tested using 3% hydrogen peroxide. Oxidase activity was tested using NNNN tetramethyl-p-phenylene diamine dihydrochloride instead of the dimethyl reagent (Table 1A).



Since the bacterium was gram-negative, further biochemical testing was carried out using an API rapid NFT test panel to identify gram-negative, heterotrophic bacteria. (Table 1B).

Results from the API rapid NFT test panel led us to identify the isolate as Pseudomonas paucimobilis. The confidence level of this identification was 98.8%.

Our isolate test results were compared to various test results expected of other yellow pigmented, non-fermenters including Xanthomonas spp.; Flavobacterium spp.; Pseudomonas cepacia; P. vesicularis; P. maltophilia and Ve group.

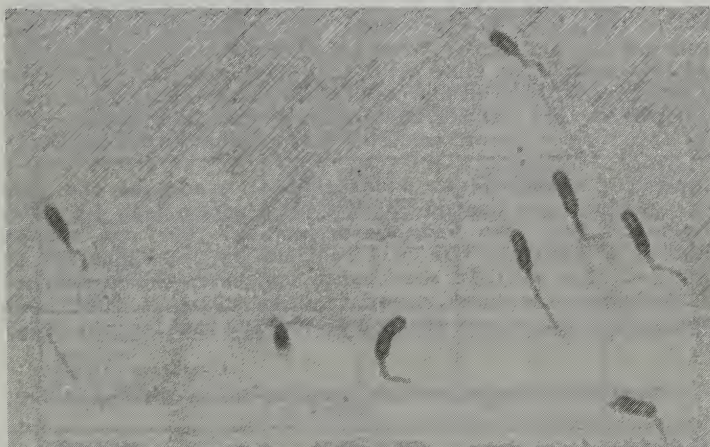


Figure 3. Flagellar Anatomy Using Mayfield and Innis Method Illustrating Single Polar Flagellum Arrangement of Redpath Isolate. Mag. 8350X.

The data showed that the test results for the Redpath isolate were identical to those expected of Pseudomonas paucimobilis but differ from other yellow pigmented non-fermenters. This confirmed the API identification (Table 1C).

We wanted to know whether an extract of the Redpath isolate pigment conformed to the observations found by Holmes et al. Holmes used the absorbance spectra of the pigment extract to differentiate Xanthomonas spp. from Pseudomonas paucimobilis.




Table 1A. Results of Bacteriological Tests on Redpath Isolate.

SCREENING TESTS:	GRAM STAIN	-
	SHAPE	ROD
	SPORE	-
	OF-GLUCOSE	-
	OXIDASE	+
	CATALASE	+
	MOTILITY	+
ADDITIONAL OFF-PANEL TESTS:	OF-LACTOSE	-
	GROWTH ON MacCONKEY	-
	FLAGELLA	SINGLE, POLAR
	PIGMENT	YELLOW (NON-DIFFUSIBLE)
	CELL ARRANGEMENT	SINGLE & PAIRS

We tried a modification of the Holmes extraction method. The isolate was grown at 30°C for 48 hours in Nutrient Broth. 4.5 gm of the suspension was then centrifuged at 2000 rpm for 10 minutes. The supernatant was pipetted off, and the centrifuge tube containing the residue was filled to original volume with sterile distilled water, slurried and spun at 2000 rpm for 20 minutes. The clear supernatant was again pipetted off and the residual water was drained.

Five ml of methanol was added (Analar grade) to the tube and residue, and heated to 50°C in a water bath for five minutes. The tube was again spun at 2000 rpm for 15 minutes to separate the cell residue and let stand for 15 minutes. The visible absorbance spectrum of the extract in methanol was then determined by using a dual beam spectrophotometer and scanned at 400-500 wavelengths (Fig. 4).

Table 1B. Confirmation Results Using API Rapid NFT.

 <b>API® Rapid NFT</b>		Ref. <u>REDPATH ISOLATE</u>	Patient <u>                    </u>
		Date <u>FEB. 26, 1987</u>	Source <u>                    </u>
		Dr. <u>                    </u>	Service <u>                    </u>

	NO <sub>2</sub>	NO <sub>3</sub>	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	NAO	MAL	GH	CAP	ADI	MT	GT	PAC	OX
24h	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	-	-	-	+	-	+
48h	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Gram	-	Flagella	+ POLAR	<b>Identification</b> <b>GOOD IDENTIFICATION</b> <b>% I.D. 98.8</b> <b>PSEUDOMONAS PAUCINOBILIS</b>
Morphology	ROD	Other		
Motility	+			

\* Rapid E, Rapid STREP, Rapid CH, STAPH Test, Rapid BTB and Rapid NFT are trademarks of API System, S.A.

Our observations of the pigment extract of Redpath isolate conformed closely with those of Holmes. Absorbance peaked in the region 447 to 452 nm and 474 to 480 nm with an inflection at 420 to 430 nm. The lack of amplitude was felt to be a result of the reduced sample size and simplified extraction methodology.

#### PSEUDOMONAS PAUCIMOBILIS

The API rapid NFT system clearly identified the isolate as Pseudomonas paucimobilis (Appendix 1). Additionally, the off panel test results (Table 1A), the comparison against other gram-negative non-fermenters (Table 1C) and absorbance spectrum pigment identification (Fig 4). were consistent with those expected of Pseudomonas paucimobilis. (Gerald L. Gilardi, 1985, pp. 48, 49), (Holmes et al 1977, pp. 136, 137), thus helping to confirm the API identification.

Table 1C. Comparison of Various Test Results of Redpath Isolate to Pseudomonas and Other Yellow pigmented non-fermenters.

TESTS	OTHER YELLOW PIGMENT NON-FERMENTERS	PSEUDOMONAS PAUCIMOBILIS	REDPATH ISOLATE
	XANTHOMONAS SPP.		
MacConkey	+	-	-
Gelatin Hydrolysis	+	-	-
Oxidase	-	+	+
	FLAVOBACTERIUM SPP.		
Motility	-	+	+
Gelatin Hydrolysis	+	-	-
MacConkey	+	-	-
	P. CEPACIA		
MacConkey	+	-	-
Lysine Decarboxylase	+	-	-
ONPG	-	+	+
	P. VESICULARIS		
ONPG	-	+	+
Mannose	-	+	+
	Ve. GROUP		
Oxidase	-	+	+
MacConkey	+	-	-
	P. MALTOPHILIA		
Lysine Decarboxylase	+	-	-
Gelatin Hydrolysis	+	-	-
MacConkey	+	-	-

Pseudomonas paucimobilis colonies on Nutrient Agar for 48 hrs. are circular, 1 mm in diameter, low convex, entire, smooth and opaque. They develop an intracellular, carotenoid (yellow) pigment which does not diffuse in Nutrient Agar nor floresce on King medium B. (Holmes et al). This yellow pigment is distinct from the brominated aryl polyenes present in Xanthomonas species (Gilardi 1985).

*P. paucimobilis* can be described as gram negative, non spore forming, aerobic, motile with singular polar flagellum, non-fermentative and yellow pigmented rods. The bacterium shows good growth on Nutrient Agar, but no growth on MacConkey. In addition:

- 0 Catalase, Cytochrome Oxidase and Deoxyribonuclease are produced,
- 0 Nitrate is not reduced to Nitrite,
- 0 Urease is not produced,
- 0 Gelatin is not liquified,
- 0 Esculin is hydrolysed
- 0 The ONPG test is positive,
- 0 Acid is produced from several carbohydrates.

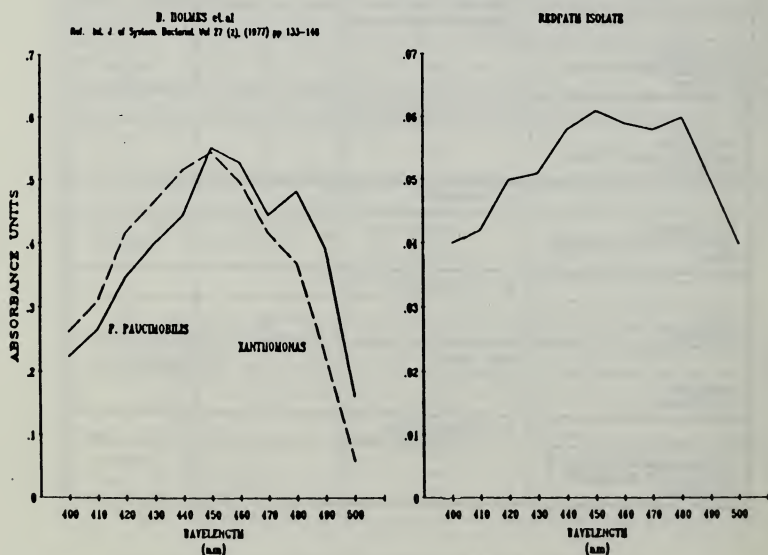


Figure 4. Absorbance Spectra of Pigments.

These characteristics are sufficient to differentiate Pseudomonas paucimobilis from other yellow pigmented non-fermenters (A. Borremass & J. Van Depiette).

The strain grew optimally at 30°C (20-37°C) but not at 5° or 42°C. Growth in Nutrient Broth produced a moderate turbidity with the formation of a pellicle and a dense deposit after 2 or 3 days (Holmes et al ).

So far 110 strains of P. paucimobilis have been identified. Its natural habitat has not been totally defined. However, Reinhardt et al indicated the organism may be found in diverse aqueous and aquatic environments.

Pseudomonas paucimobilis is listed as a non-fermenter, however it does assimilate sucrose and fructose via oxidation which results in the production of acid.

Table 2A. Test Results of Redpath Isolate on Nutrient Agar at Various pH and Temperature.

TEMPERATURE RANGE	pH	24 HR INCUBATION	96 HR INCUBATION
ROOM TEMP. (20°C)	3.9	NG	NG
	4.9	SG	G
	6.7	G	G
	6.9	G	G
	8.4	SG	G
30°C	3.9	NG	NG
	4.9	GG	GG
	6.7	GG	GG
	6.9	GG	GG
	8.4	G	G
35°C	3.9	NG	NG
	4.9	GG	GG
	6.7	GG	GG
	6.9	GG	GG
	8.4	G	G
42°C	3.9	NG	NG
	4.9	NG	NG
	6.7	NG	NG
	6.9	NG	NG
	8.4	NG	NG
50°C	3.9	NG	NG
	4.9	NG	NG
	6.7	NG	NG
	6.9	NG	NG
	8.4	NG	NG

Note:

G GROWTH  
GG GOOD GROWTH  
SG SLIGHT GROWTH  
NG NO GROWTH



Although the amount of acid produced is not of the same magnitude as that produced by fermentation, its action on sucrose may affect final product stability. Some species in the *Pseudomonas* genus have been known to produce a variety of products that deleteriously affect flavour. Also, their aerobic tendencies enable them to grow rapidly and produce oxidized products and slime at the surface of foods. However, the last two tendencies have not yet been related directly to *Pseudomonas paucimobilis*.

#### P. PAUCIMOBILIS IN THE REFINERY ENVIRONMENT

Different groups of bacteria have different temperatures, ranges and optimal temperatures for growth. In fact temperature sensitivity is one of the most important factors influencing the activity of bacterial enzymes. Because bacteria lack mechanisms to conserve or dissipate heat generated by metabolism, their enzyme systems are directly affected by ambient temperatures. Some organisms have a wide range for growth while others rapidly die at temperatures outside a normal growth range. In all cases, enzymatic reactions progress quickly at optimum temperature but become inactive above or below their

Table 2B. Results of Exposure of Redpath Isolate to 90°C for Various Periods.

EXPOSURE TEMP (90°C)	EXPOSURE-TIME										
	BLK	OB	10s	20s	40s	1m	2m	4m	6m	8m	OA
SET # 1	-	+	+	+	+	+	-	-	-	-	+
SET # 2	-	+	+	+	+	+	-	-	-	-	+

BLK (BLANK) NUTRIENT BROTH WITH NO INNOCULUM

OB (OBSERVATION BEFORE) NUTRIENT BROTH WITH 1 DROP OF INNOCULUM (NO EXPOSURE) AT BEGINNING OF EXPERIMENT

OA (OBSERVATION AFTER) NUTRIENT BROTH WITH 1 DROP OF INNOCULUM (NO EXPOSURE) AT END OF EXPERIMENT

s SECONDS

m MINUTES

+

POSITIVE GROWTH

- NO GROWTH



temperature range. Biochemical tests are usually carried out at the optimal growth temperature of the isolate. Aside from temperature, the hydrogen ion concentration of an organism's environment also plays a significant role in its growth, since pH limits the activity of enzymes with which an organism is able to synthesize new protoplasm. Like temperature, there is also an optimum concentration of hydrogen ions for maximum growth (Benson).

We initiated further studies to relate optimum growth conditions to our refinery operating conditions. The effects of the temperature (growth and thermal endurance) and pH of our process conditions were studied (Table 2A).

Results indicated that the organism did not grow at pHs below 4.9, and growth was slowed at pH 8.4. This explains why the organism was never found in the invert process and storage system. Secondly, optimum growth was achieved at temperatures of 30° and 35°C, while growth at 20°C was slowed. At 42°C and above, growth was totally inhibited. This agreed with Holmes observations of growth at 37°C, but not at 5°C or 42°C, with an optimum temperature of about 30°C.

To check the recoverability of the organism, isolates previously incubated at 42°C and 50°C were re-incubated at optimum temperature of 30°C. After 48 hours incubation, plates initially incubated at 42°C showed good growth, while plates initially incubated at 50°C showed none. This indicated that the organism's recoverability was limited by exposure to higher temperature.

We then studied the thermal endurance of the isolate. The thermal-death time tube method was used at a temperature of 90°C (pasteurizer conditions). Results are listed in Table 2B. Complete kill was achieved in less than 2 minutes. Owing to the thermal lag inherent in this experiment a kill in less than 1 minute was deemed feasible.

Our study established the pH and temperature characteristic of the organism enabling us to identify and monitor potential areas of contamination. This information was later used to effect interim control.

#### **SOURCE OF CONTAMINATION**

In an attempt to determine the source of the isolate an intensive sampling of the system was carried out. We monitored incoming raw sugar, liquid sugar process, liquid loads and loading sources as well as the general refinery

environment. Plate count results of the first two areas are outlined in Tables 3A and 3B.

Our results indicated that the bacteria did not originate with raw sugar. An evaluation of the liquid sugar process indicated the bacteria is sporadically present at low levels in certain areas of the product stream. However, a significant trend was not discernable.

Results from environment checks (floors, walls, filters, etc.) also indicated the presence of the organism but the results were again, not significant.

Despite what appeared to be a random appearance of the bacteria, a review of the liquid load and storage tanks data did reveal a pattern. It was now possible, by

Table 3A. Results of Tests for the Presence of *P. Paucimobilis* in Raw Sugar Cargos, April - December, 1987.

ORIGIN OF RAW SUGAR	PSEUDOMONAS PAUCIMOBILIS (COUNTS/GM)
CUBA	0
GUYANA	0
MAURITIUS	0
QUEENSLAND	0
SWAZILAND	0
ZIMBABWE	0

overlaying liquid load results with the storage tanks results, to determine that cross contamination was occurring in the system. We found that a truck which had been sanitized did not have any counts when repeatedly loaded from storage except when loaded first from one particular tank. When a truck was first loaded from this tank, its subsequent loads from all other tanks were also positive for *P. paucimobilis*. What we thought was random contamination really came from a single area, storage tank E. We still had to determine why the test results for Tank E differed from those for A and B.

We found that unlike A and B, storage tank E serves a dual function. Firstly, it is used to store a non-standard product called Low Colour Sucrose. Secondly, it serves as a backup tank during heavy production runs for normal sucrose product.

Low colour sucrose production required certain process changes, so it was felt that something was occurring in these runs to cause the presence of P. paucimobilis. We monitored a low colour sucrose run and obtained the results noted in Table 3C. Although some P. paucimobilis was present, the production process was not found to be a main source of the contaminant.

Table 3B. Results of Tests for the Presence of P. Paucimobilis in Process Liquid Sugar Stream, February, 1988.

TANK NO.	DESCRIPTION OF REFINERY TANKS	BRIX	TEMP ( °C )	PS.PAUCIMOBILIS /10 GM TEST#1	TEST#2
T.O.C.	BEFORE CHAR	65	75-80°	0	0
FINE LIQUOR	AFTER CHAR	65	75-80°	0	0
588	FILTERED LIQ.	65	75-80°	3	3
5891	SOCK FILTERS		50°	4	4
5901	MONOBEDS	58	55°	31	35
	RESIN BED	65	75-80°	NS	0
5911	DEASHED LIQ. MIXING TANK	58	50-55°	0	0
5504	LIQ.SUGAR PREMELTER	78	80-90°	1	0
5500	LIQ. SUGAR MELTER	78	80-90°	0	0
556	PRESS SUPPLY	67.5	80-90°	0	0
586	AUTOJET FILTER	67.5	80-90°	0	0
591	SUCROSE COOLER SUPPLY	67.5	40-50°	0	0
560	SUCROSE COOLER	67.5	40°	1	0

NOTE :

NS NOT SAMPLED

In the meantime, the results of environmental sampling (swabs, air-plates, filter units) revealed sporadic low levels of Pseudomonas paucimobilis around the truck handling area and liquid storage environment. However, subsequent sampling of storage tank E immediately after low colour production did reveal abnormally high counts of P. paucimobilis.

How was it possible to have this magnitude of counts when the process results were so insignificant? We focused our attention on tank E to locate a specific source acting as an inoculum in the system. Only such a possibility could explain the extreme counts over such a short time period.

Table 3C. Results of Tests for the Presence of P. Paucimobilis in Process "Low Colour" Sugar Stream, March - May, 1988.

TANK	TEST# 1 4/3/88	TEST# 2 22/3/88				TEST# 3 7/4/88	TEST# 4 27/4/88	TEST# 5 (COUNT/ 26/5/88 10GM)	
	T C	T	C	T	C	T	C	T	C
556	2p 0	12n 0	9p 0	4p 0	11a 0	12n 0	12n 0		
	3p 0	1p 0	10p 0	5p 1	12n 0	6p 0	6p 0		
	4p 0	2p 0	11p 0	6p 0	1p 0	7p 0	7p 0		
	5p 0	3p 0	12 0	7p 0	2p 0	8p 0	8p 0		
	6p 0	4p 0	1a 0	12 0	3p 0	9p 0	9p 0		
	7p 0	5p 0	2a 0	1a 0	4p 0	10p 0	10p 0		
		6p 0	3a 0	2a 0	5p 0	11p 0	11p 0		
		7p 0	4a 0	3a 0	6p 0	12 0	12 0		
560	2p 2	12n 1	9p 0	4p 0	11a 0	12n 0	12n 0		
	3p 0	1p 0	10p 1	5p 0	12n 0	6p 1	6p 1		
	5p 0	3p 2	12 1	7p 0	2p 0	8p 0	8p 0		
	6p 0	4p 12	1a 11	12 2	3p 0	9p 0	9p 0		
	7p 0	5p 0	2a 7	1a 2	4p 0	10p 1	10p 1		
		6p 4	3a 2	2a 1	5p 0	11p 1	11p 1		
		7p 2	4a 2	3a 1	6p 0	12 0	12 0		
Es.	Es. ADD	Es. ADD		Es. ADD		Es. ADD		Es. ADD	
	4p 0	3p 0		7p 0		2p 0		12n 0	
	5p 0	4p 0		8p 0		3p 0		1p 0	
	6p 0	5p 0				4p 0		2p 0	
						5p 0		3p 0	
						6p 0			
TANK "E"	7/3/88 126	22/3/88 1		7/4/88 0		27/4/88 190		26/5/88 0	

NOTE :

T TIME

C COUNT/10 GM

Es. ECOSORB



An investigation into the use of F tank revealed that the tank was only used intermittently, and it was, therefore, not regularly sampled for microbiology when it was deemed "empty". We took tank E off the production stream and examined it.

Samples from within the tank (residual product, walls, floors) revealed high levels of P. paucimobilis. A visual inspection of the tank interior found that it differed in construction from other storage tanks. It is a newer tank with a depressed area, or sump, while older tanks have flat bottoms (Fig.5). It was determined that while flat bottom tanks can easily be completely emptied, the sump in tank E was never fully drained prior to regular sanitation, thus allowing some sucrose to remain in the tank.

We drained the sump area completely, using a lower outlet valve, and resanitized the tank with steam using our established procedure. Microbiology tests of the resanitized tank showed no evidence of Pseudomonas paucimobilis. We concluded that the regular sanitizing procedure for storage tanks did not take into consideration the anomaly in tank E, and could possibly lead to unsatisfactory sanitation.

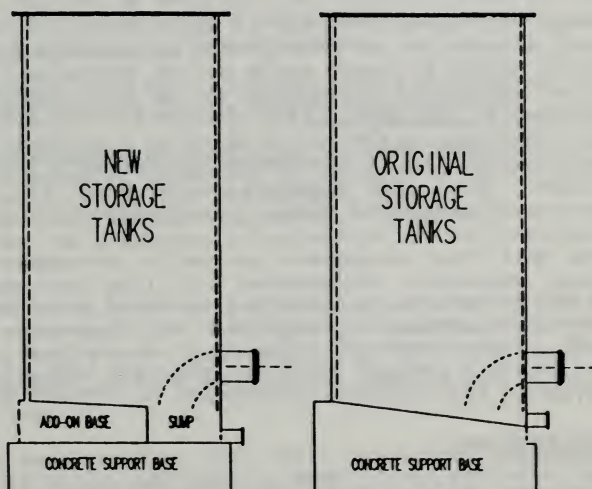


Figure 5. Different Base Configurations of Storage Tanks.



When incomplete cleaning was followed by a sufficiently long dormant period, the sump likely acted as a breeding ground for bacteria contamination. When the tank was again filled, this bacteria then acted as an inoculum to the incoming product causing relatively high counts over a short time frame.

Because of these results, our sanitizing procedure was altered to ensure predraining of the tank sump area. Monitoring of the system was continued after these procedural changes were made, and no further trace of Pseudomonas paucimobilis was noted.

We then eliminated the organism from the Liquid Distribution system using a procedure called "Burn Out". This involved circulating high temperature product through the lines in a loop fashion starting from the storage tanks to the loading bay and back. Steam sanitation remained an effective and non-additive control measure. However various biocides were also found to be effective, (Sodium Dichloroisocyanurate, 1-2% acetic acid, sodium paratoluene sulfoncholoramide) and their inclusion in washing solutions eliminated the organism from all external surfaces.

#### CONTROL AND SUMMARY

Although our study resulted in the identification and virtual elimination of the species P. paucimobilis, the specific portal of entry could not be determined. Many parts of the system are exposed to the environment, and, as observed P. paucimobilis was present in virtually every area. Additionally the use of water as a diluent in the preparation of liquid sucrose could act as a source of inoculum. Thus, the organism could enter the system sporadically at several points throughout the process.

It was only when inadequate sanitation procedures left residual materials undisturbed for long periods, did significant bacterial contamination occur. By adopting sanitation methods appropriate to the new tank configuration, and by increasing the scope of our monitoring activities, the problem with P. paucimobilis was eliminated.

#### ACKNOWLEDGEMENTS

The authors would like to thank Gary Horsnell for his taxonomic analysis, technical advice and helpful insights; Richard Chong-Kit for his help in preparing the photomicrographs; and Andrew Ho for his assistance in setting up the figures and tables.

#### APPENDIX A: P. PAUCIMOBILIS CHARACTERISTICS

- Defn.: Paucimobilis - indicates that only a few cells in a population may be motile.  
< ADJ - Paucus FEW; < ADJ Mobilis Mobile  
Paucimobilis - intended to mean few cells mobile.
- Previously referred to as a Pseudomonas-like bacterium, Group II K Biotype I & II, by Doudoroff and Palleroni (1974)
- Was differentiated by Holmes et. al (1977) into new species Paucimobilis strain CL1/70.
- Further work by Owen and Jackman (1982) suggests that evidence from rRNA cistron comparisons and cellular fatty acid profiles indicate this species not to be an authentic member of the genus Pseudomonas and that P. paucimobilis may justify inclusion in a new genus (Gilardi, 1985).
- The natural habitat of Pseudomonas paucimobilis has not been totally defined; however, Reinhardt et al (1979) indicated the organism may be found in diverse aqueous and aquatic environments.
- Little information is available on isolation from the soil. Experiments by Elliot et al (1983) indicate the soil is a good support medium for this bacteria.
- These organisms have been isolated from a variety of clinical specimens and sources in the hospital environment.
- Recently Pseudomonas paucimobilis has been reported to be a causative agent of infection in humans, including a leg ulcer secondary to injury and several reports of bacteraemia. Further investigation is proposed to characterize the potential invasiveness of this pathogen (Smalley, 1982).
- Further profiling by Smalley (1982), using the API ZYM system tested 19 enzymes for in chromogenic dependent substrate reactions resulting in 16 positive for Pseudomonas. The author postulated that some of these enzymes might be potentially related to Pseudomonas paucimobilis virulence (Gilardi, 1985).
- Literature data suggests that the virulence of Pseudomonas paucimobilis is low and that in most of the patients the bacteria are introduced by medical manipulation (Borremans and Vandepitte).

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## DISCUSSION

Question: Do you continue to pasteurize your product liquid sugar now that you have solved this infection problem?

Dewar: We no longer pasteurize the final product, but we still maintain the sanitizing procedures which include "burn out" (pasteurizing) of the distribution system.

Question: Did you say that this organism also produces off-flavors?

Dewar: We haven't gotten any data on that. I mentioned that some of the *Pseudomonas* species produce a variety of products that may affect flavor. However, we did not specifically evaluate this relationship.

Question: Have you noticed any off-flavors or odors in your product?

Dewar: No, there did not appear to be any changes to the product other than the higher micro counts. Of course, our priority was to eliminate the organism before it could cause further problems.



## SUCROSE LOSSES IN SUGAR AND FOOD PROCESSING - EFFECTS OF IMPURITIES

G.N. Richards

Wood Chemistry Laboratory, University of Montana, Missoula, MT 59812

### INTRODUCTION

Pure, crystalline sucrose is relatively stable to heat. E.g. it is difficult to measure any chemical reaction or loss of purity when pure sucrose is heated at 150°C for many hours. Similarly, it is usually assumed that pure sucrose in pure water is relatively stable to boiling. This paper addresses the above two situations separately. The chemistry involved is based on research published in a series of papers from the James Cook University of North Queensland in Australia and more recently from the Wood Chemistry Laboratory of the University of Montana (Moody and Richards, 1983; Richards, 1986; Lowary and Richards, 1988). This research has elucidated the mechanisms summarized in Figure 1 for thermal degradation of sucrose. The major intention of this paper is to relate this detailed chemistry to the "real life" world of sugar and food processing. In such situations the sucrose is rarely pure and we must therefore deal with the very dramatic and specific effects which we have recently shown to be associated with the presence of small amounts of impurities such as salts and reducing sugars.

### DEGRADATIONS OF AMORPHOUS SUCROSE

To consider first the case of amorphous sucrose, with little or no water content, this may occur in the burning of cane at harvest, where dried down juice may produce amorphous sucrose, which as a result of fire temperatures is likely to yield kestoses (see below). Molasses are often subjected to long periods at relatively high temperatures and this situation will result in similar chemistry to that described below. Finally, in many food process operations, sucrose is initially dissolved in water, dried down during the process (e.g. baking), often to produce amorphous sucrose, which may be exposed to the level of thermal treatments and chemistry described below. Some of the results on such systems have been described previously (Richards, 1986).

In preliminary experiments it was observed that powdered pure sucrose crystals would survive for many hours at (say) 150°C

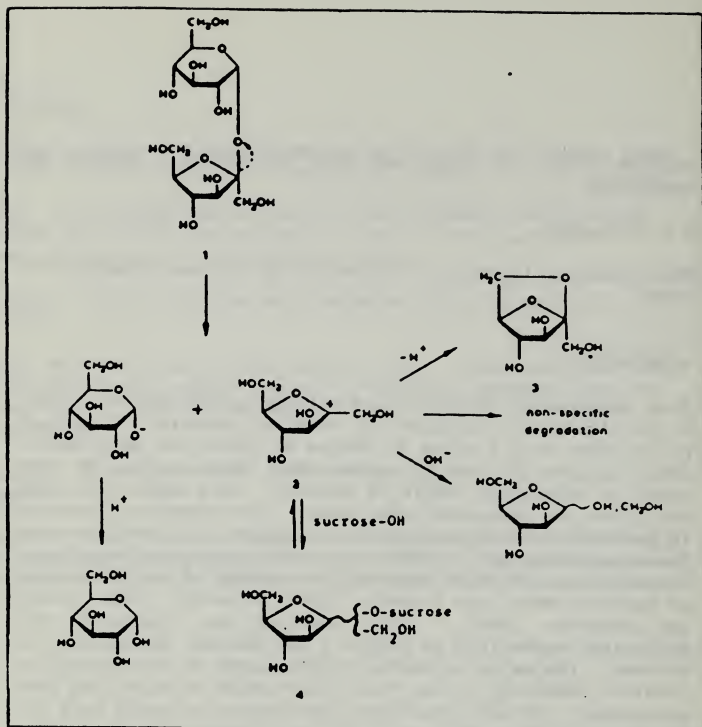


Figure 1. Mechanism of thermal degradation of sucrose.

without any detectable degradation beyond slight darkening and evidently this stability is associated with relative absence of molecular mobility in the crystalline lattice. In practice, however, sucrose will not always be crystalline when subjected to heat. An experimental procedure was therefore designed (Richards, 1986) to produce amorphous sucrose (a melt) at temperatures well below its crystal melting point and under these conditions the sucrose degrades very much more rapidly than the crystalline material. The loss of sucrose in the melts is shown in Figure 2. The rates are too rapid for meaningful analysis at the higher temperatures, but at temperatures of 150°C and lower the most dramatic conclusion from these curves is that there is an unequivocal lag phase in the degradation.

The products of the degradation of pure sucrose at 135°C are shown in Figure 3. Glucose is formed in relatively high yield, while fructose is always produced in lesser amount and is itself more subject to further thermal degradation than glucose, thus producing the maximum in the fructose curve. Trisaccharides (kestoses) are formed in smaller yield and traces of anhydrofructose (Poncini and Richards, 1978; Richards and Shafizadeh, 1978) and of disaccharides other than sucrose are also observed but are not plotted on Figure 3.

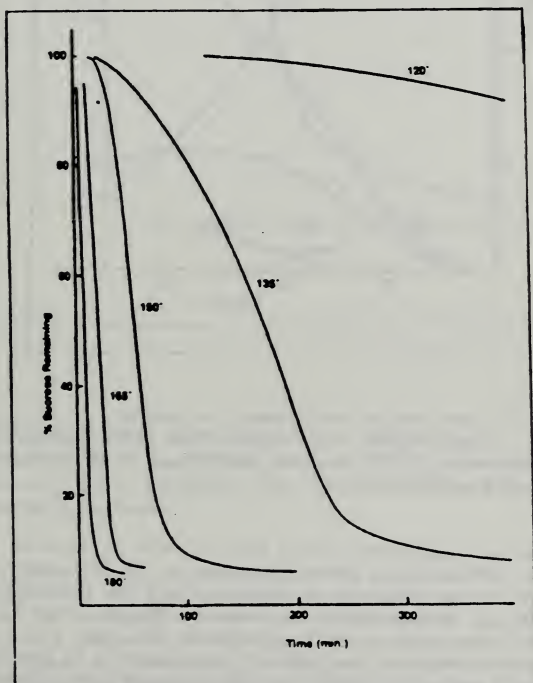


Figure 2. Degradation of sucrose melts at various temperatures.

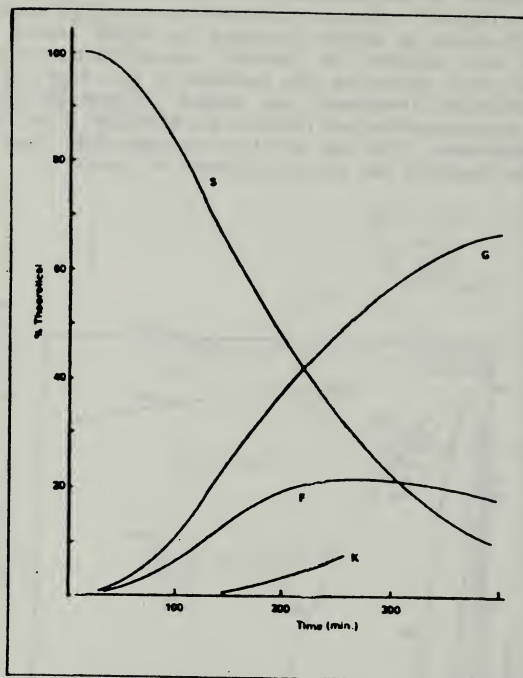


Figure 3. Degradation of sucrose melt and formation of major products at 135°C: S = sucrose, G = glucose, F = fructose and K = kestoses.

The effects of impurities on the rate of loss of sucrose were studied at 120°C and are shown in Figure 4. Of the impurities used, only sodium carbonate had the effect of reducing the rate of thermal decomposition of sucrose. The presence of 10% glucose in the sucrose melt shortened the lag phase and accelerated the degradation. With 5% glucose plus 5% fructose, both effects were increased and 10% fructose (not shown in Figure 4) was even more potent in accelerating the degradation. Addition of a neutral salt (sodium chloride) to the sucrose melt was also remarkably effective, at less than 1% concentration, in reducing the lag phase and accelerating the decomposition.

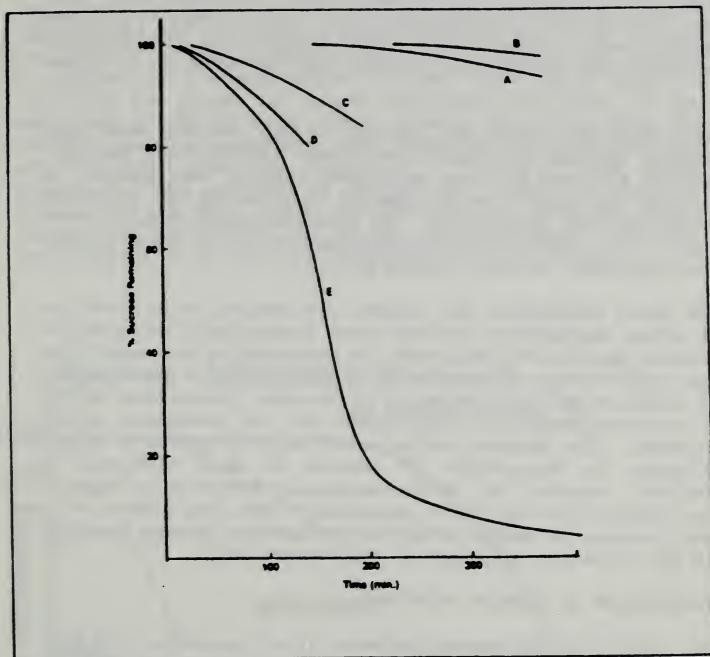


Figure 4. Effect of impurities on the rate of degradation of sucrose melts at 120°C: A = pure sucrose, B = sodium carbonate (0.04 mole/mole sucrose) C = glucose (10%), D = glucose (5%) + fructose (5%) and E = sodium chloride (0.05 mole/mole sucrose).

The probable explanation of the lag phase is that the initial degradation of pure sucrose is extremely slow, in fact too slow to be detected by the methods used in this study. However, traces of initial degradation products formed during this phase may themselves be subject to more rapid degradation reactions and some products of such secondary reactions may be acidic. Such products (e.g. formic, levulinic acids) could be extremely small in amount, yet could result in protonation of sucrose and hence in an increasing rate of degradation. As the degradation proceeds the carbocation (2) would be increasingly produced and would partly undergo non-specific



degradation reactions to produce more acid catalysts. The same non-specific degradations would also include reactions which generate water which would provide the hydrogen and hydroxyl ions required for further reaction as shown in Figure 1.

When reducing sugars are present in the sucrose melt they undergo thermal degradation much more rapidly than sucrose. Because fructose degrades much more rapidly than glucose it is much more potent as an impurity in reducing the lag phase in the sucrose degradation. In confirmation of this, it was found that a fructose melt, after heating at 150°C for 30 min, when dissolved in water showed pH 4.5.

The above hypotheses all require the initial slow formation of acidic degradation products which increasingly catalyze the sucrose degradation and result in accelerating decomposition. The stabilization of sucrose by the presence of a small amount of sodium carbonate (Figure 4) is therefore interpreted as due to neutralization of the traces of the secondary acidic products. The dramatic influence of very low levels of sodium chloride in degradation of sucrose is more difficult to explain. However, all of the mechanisms involved in Figure 1 are heterolytic and it is conceivable that the effects of a small amount of sodium chloride may operate through increase in the dielectric constant of the sucrose melt.

#### DEGRADATION IN AQUEOUS SOLUTIONS

The hydrolysis of sucrose by water (i.e. inversion) has been extensively studied, especially with added acid catalysts in dilute solution. However, in sucrose processing, sucrose is most frequently exposed to heat in very concentrated aqueous solution at about neutral pH. The evaporation of solutions to crystallization during milling and refining is an obvious example where even a low level of hydrolysis can have important economic impact. In food processes also, we frequently find examples of exposure of concentrated aqueous solution of sucrose to high temperatures for significant periods. In all of these circumstances the sucrose solution generally contains impurities such as reducing sugars and inorganic salts and it is this type of system which we have now studied.

Figure 5 shows the rate of loss of sucrose at 100°C in a solution containing 20 g of sucrose and 7.5 g of rigorously deionized water. The rate is measured by HPLC against an internal standard (ethanol) added to a sample of the solution after heating for the required time in a sealed glass tube. As with the thermal degradation of amorphous sucrose, a lag phase is observed in sucrose loss and we postulate the same

type of explanation. That is, that the initial rate of hydrolysis of sucrose by water is extremely slow, but finite. The products are fructose and glucose and especially the former degrades relatively rapidly under these conditions to produce a mixture of products which includes some acids such as levulinic and formic (of course hydroxymethylfurfural is the major degradation product). These minor acid products form the initial hydrolysis products, then induce the autocatalytic form of the curve shown in Figure 5. To verify the above hypothesis, the addition of 10% glucose (based on sucrose) reduces the lag phase, while the same amount of fructose almost removes the lag (Figure 5). In the same experiment, fructose was observed to be lost much more rapidly than glucose.

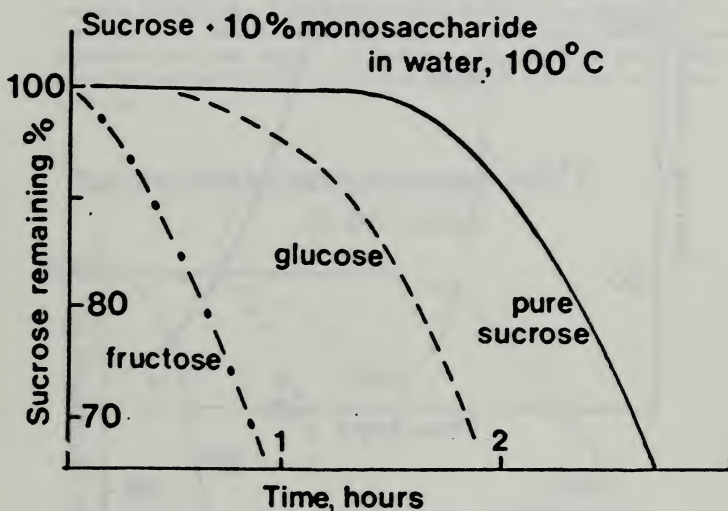


Figure 5. Degradation of sucrose in water at 100°C; effect of added glucose and fructose.

The effect of a small amount of a weak base on sucrose hydrolysis at 100°C is shown in Figure 6, where 0.05 mole of sodium carbonate per mole of sucrose is seen to confer complete stability (within the accuracy of the experiment) on the sucrose for more than four hours. This is interpreted as due to neutralization of secondary acidic degradation

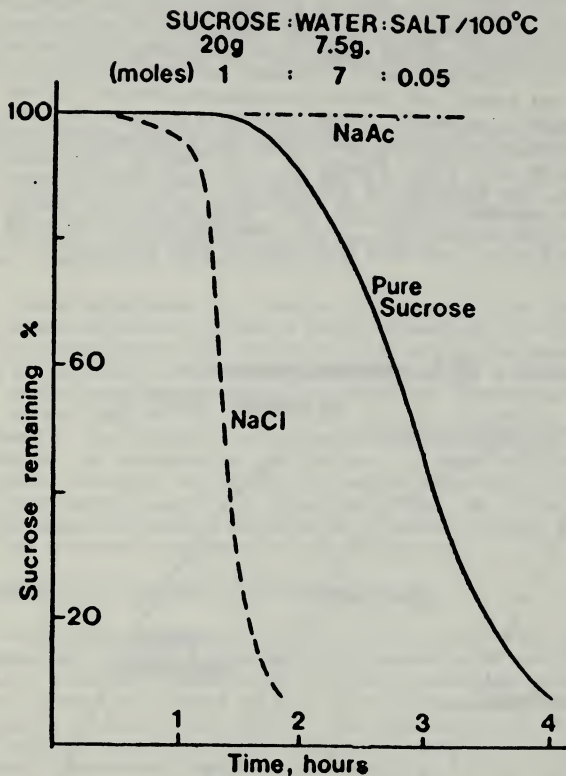


Figure 6. Degradation of sucrose in water at 100°C; effect of sodium acetate and sodium chloride.

products which would form from any traces of primary hydrolysis products. The effect of sodium chloride on the sucrose hydrolysis is also shown in Figure 6. This is observed as a dramatic shortening of the lag phase in sucrose loss (i.e. an acceleration of the hydrolysis). This effect is produced by a ratio of only one mole of sodium chloride to 20 moles of sucrose. There are two possible types of explanation for this effect. Either the sodium chloride accelerates the initial

slow hydrolysis of sucrose and thus increases the initial rate of formation of primary products and hence the rate of formation of acidic secondary degradation products, or alternatively, the sodium chloride accelerates the rate of degradation of primary products (glucose and fructose) to acids. It is possible that both effects operate, and on the present evidence we are not able to reach a definite conclusion, but in general the first interpretation is favored at this stage.

The influence of other salts is shown in Figure 7. Cations have been chosen which are common and often abundant in sucrose processing; the anion is chloride throughout and the mole ratio of salt to sucrose has been maintained at 1:20. It is evident that calcium ions are more effective than sodium and that magnesium ions are much more effective in accelerating the sucrose hydrolysis. The other alkali metal chlorides were also studied, but within the accuracy of our experiments, they produced the same effect as sodium. Preliminary experiments also indicated that use of other halides produced only small effects.

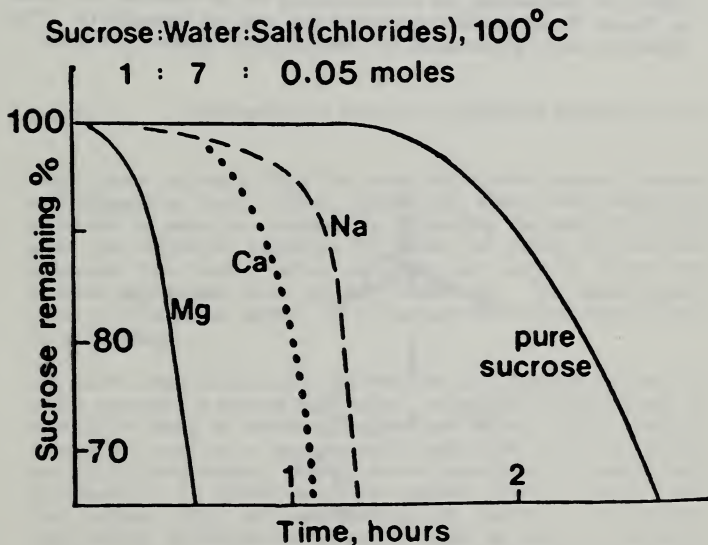


Figure 7. Degradation of sucrose in water at 100°C; effect of salts.

The explanation of the influence of magnesium ions on sucrose hydrolysis must be speculative at this stage. The first step in hydrolysis of sucrose is most probably the scission of the oxonium ion shown in Figure 8, where G is glucose and F is fructose (other oxonium ions will form at alcohol hydroxyl groups). Any effect which increases the concentration of oxonium ion will increase the rate of hydrolysis and of course this is the basis of acid catalysis of sucrose inversion. One of the most familiar differences or trends between sodium, calcium and magnesium salts is the increasing tendency of the cation to form stable hydrates and this provides a possible explanation for the effects shown in Figure 7. In this system, it should be noted that the number of potential hydrogen-bonding sites of sucrose molecules exceeds the number of water molecules and that there will be competition for water molecules between sucrose and the cations. The magnesium ions are likely to be especially effective in this competition and the water molecules which are hydrated to magnesium will have oxygen-hydrogen bonds which are more polarized (i.e. more acidic) than free water molecules. Thus, as shown in Figure 9, there will be an increased tendency for transfer of hydrogen ion from a hydrated water molecule to any other electron donor such as the glycosidic oxygen of sucrose. This will have the effect of increasing the concentration of the oxonium ion shown in Figure 8 and hence increasing the rate of hydrolysis. The effect is most pronounced in concentrated aqueous solution

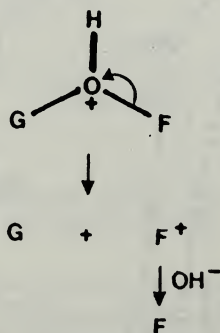


Figure 8. Hydrolysis of sucrose.



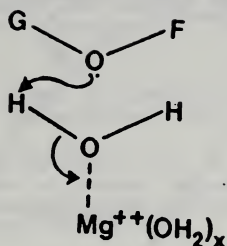


Figure 9. Protonation of sucrose by hydrated magnesium ion.

where there is competition for water molecules between sucrose and magnesium ions. Thus, Figure 10 shows that magnesium chloride is much less potent in increasing sucrose hydrolysis when the solution contains 50 moles of water per sucrose molecule than with 7 moles of water per sucrose, while the sucrose:magnesium ratio is kept constant. The same figure shows little or no effect of variation of sucrose:water ratio in pure water.

The above experiments are relevant to any sucrose process in which sucrose is heated with water, especially in presence of impurities such as reducing sugars and salts. They indicate a need for particular concern when magnesium ions are present in significant amount, as may occur especially in sugar beet processing. It should be noted however, that in "real life" the anions will not necessarily be halide. In juices especially, carboxylic acid anions such as acetate, lactate, citrate, etc. are present and these (and anions of any other weak acid) may exert alkaline buffering effects which will effectively stabilize the sucrose towards hydrolysis in the same way as the sodium carbonate shown in Figure 6.

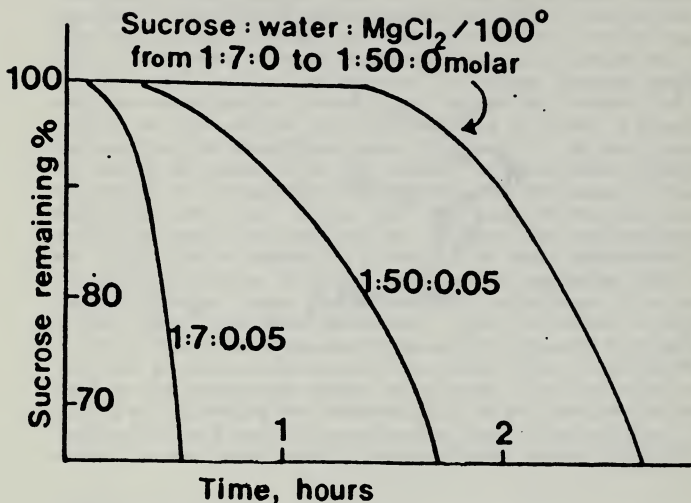


Figure 10. Influence of water content on catalysis of sucrose hydrolysis by magnesium chloride.

#### ACKNOWLEDGEMENTS

Part of the work described above was carried out at James Cook University of North Queensland with the experimental assistance of G.B. Hawes and J.G. Kelly and financial support from the Sugar Research Institute, Mackay. The remainder of the experimental results, especially those involving aqueous solution, owe much to the experimental assistance of T.L. Lowary.

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## DISCUSSION

Question: What do you mean by pure sucrose? Also, where did you find amorphous sucrose? I know a lot of work done by Niedieck and others in Germany at Karlsruhe University, where they did not find amorphous sucrose. Only when milling sugar to icing sugar does there exist amorphous sucrose.

Richards: Amorphous sucrose is made by taking a concentrated aqueous solution and flashing off the water quickly. Then you get a glass containing nothing but sucrose and no water. There is no doubt that the glass is amorphous. It is a common way to stop things from crystallizing--you remove the solvent quickly, and the molecules don't have time to organize into a lattice.

In the work on these glasses or melts, we always inspect carefully and throw out any sample that is dubious. It is visibly inspected to be sure it is a clear, colorless glass with no nucleus for crystallization. We have confirmed the anisotropic structure of the glasses with a polarizing microscope.

Question: This is a comment that related to the first question about your definition of pure sucrose. In my company, we have been very concerned about the color of sugar. We have established that if you examine individual crystals, you will find a considerable difference in their behavior when they are heated at high temperatures. Some crystals, as the work of Prof. Mantovani in Italy has shown, contain micro-inclusions, almost at the molecular level of impurities. When you heat such crystals at about 105°C for a period of hours, maybe 8 hours, you can determine very clearly the browning reactions inside those. This observation is of use to people who wish to have a simple test to determine which crystals have higher color inclusions than others. I wonder, therefore, what you understand by "pure" sucrose?

Richards: This is defined in our papers as Baker's Analyzed Reagent. We don't investigate the purity any further, but I have seen papers in the literature describing extensive chemical reactions from supposed pure sucrose, which wasn't defined in most papers, saying that the sucrose was heated at 150°C for about 8 hours and then they isolated kestoses. We have heated Baker's Analyzed Reagent sucrose overnight at 150°C and there is only a slight coloring. Analysis by HPLC, which gives us about  $\pm 1\%$  accuracy, shows it is still 100% sucrose. It is crystalline and stable.

There is no doubt whatever that for the same sucrose molecular analyzing better than 99% sucrose by HPLC--on the one hand in a non-crystalline glass and on the other hand crystalline--the amorphous sample degrades quickly on heating (e.g. at 150°C) and the crystalline one is very much more stable.



## COMPOSITION OF DEXTRAN IN RAW SUGAR AND SUGARCANE JUICE

E. J. Roberts, M. A. Clarke, M. A. Godshall, and T.B.T. To

Sugar Processing Research, Inc.

### INTRODUCTION

During our work in the development of the copper method for dextran, it was found that the copper precipitate, upon hydrolysis, sometimes contained small amounts of other sugars in addition to glucose. Arabinose and galactose are associated with ISP, and xylose with bagasse. There was always observed an unusually large amount of mannose. If the dextran was precipitated at room temperature and well washed, it contained no ISP sugars, but did contain small amounts of mannose.

This led us to suspect that the wild organisms from field soil can produce a polysaccharide that includes some mannose in its structure. Two possibilities are: enzymes that convert glucose to mannose which becomes part of the dextran chains, probably as end groups; or enzymes that convert the reducing end groups of the dextran to mannose. No mannose was found in dextran produced by pure strains of Leuconostoc mesenteroides. All samples of dextran isolated from raw sugar or cane juice contained small amounts of mannose, ranging from 1.5% to 3.0%.

This paper describes the evidence obtained to date indicating that the mannose is covalently attached to the dextran.

### EXPERIMENTAL

#### Isolation of Cane Dextran

An amount of 500 g of raw sugar containing 900 ppm of dextran was dissolved in water and diluted to 1000 ml. Then 1g of filter aid and 3000 ml of alcohol were added. The solution was allowed to stand 1 hr to allow the precipitate to settle. The supernatant liquid was decanted and the precipitate was filtered off on a filter precoated with a filter aid mat. The precipitate was washed several times with 80% V/V ethanol. The dry precipitate was suspended in 50 ml of water, heated to boiling and filtered. The precipitate was again boiled in 50 ml of water and filtered. The combined filtrates were cooled and 100 ml of 2.5N sodium hydroxide saturated with sodium sulfate, 1 g of filter aid and 200 ml of copper reagent containing 24 g of sodium sulfate were added. The

copper reagent was prepared by diluting 100 ml of copper stock solution (containing 3.0 g of copper sulfate and 30 g of sodium citrate per liter) with 100 ml of water. The solution was allowed to stand at room temperature for about 30 min. The precipitate was filtered on a filter aid mat and was washed several times with 25 ml portions of a wash solution composed of 100 ml of water, 20 ml of 2.5N sodium hydroxide saturated with sodium sulfate and 20 ml of copper reagent (Roberts Copper Method, Clarke and Godshall, 1988).

After it was sucked dry on the filter, the precipitate was slurried in 50 ml of water and an excess of ion exchange resin (CG-50, 100-200 mesh) in the hydrogen form was added and stirred for about 30 min. The solid material was filtered off on a filter aid mat and washed several times with water.

The combined filtrate and washings were dialyzed for 100 hours in a 12,000 MW cut off bag. The contents of the bag were evaporated to a small volume and freeze dried yielding 0.35 g of dextran.

#### Hydrolysis of Cane Dextran

The cane dextran was hydrolyzed with 2N sulfuric acid as described by Roberts and Godshall (1978), or with trifluoroacetic acid as described by Fengel and Wegner (1979). When sulfuric acid was used, 0.2 g of the dextran was dissolved in 10 ml of 2N sulfuric acid and boiled under reflux for 4 hours. The acid was neutralized with barium carbonate. The salts were removed by filtration. The filtrate was concentrated under reduced pressure and freeze dried. When trifluoroacetic acid was used, 0.2 g of the dextran was dissolved in 10 ml of 2N trifluoroacetic acid and boiled under reflux for 4 hours. The solution was evaporated to dryness under reduced pressure. Then 10 ml of water was added and again evaporated to dryness. The solid material was dissolved in 10 ml of water and freeze dried. The yield of each hydrolyzate was 0.2 g.

#### Acetolysis of Cane Dextran

Cane dextran (0.2 g) was dissolved in a mixture of 2.5 ml of acetic anhydride, 1.5 ml of acetic acid and 4 drops of concentrated sulfuric acid. The acetolysis was carried out as described by Wolfman and Franks (1962), and yielded 0.23 g of product.

#### Methanolysis of Cane Dextran

Cane dextran (0.3 g) was methanolized by the procedure of Roberts et al. (1987). The dextran was dissolved in 5 ml of 72% methanolic sulfuric acid. The solution was then diluted with 300 ml of absolute methanol; 5 g of Drierite was added and the solution was boiled under reflux for 12 hrs. The solution was evaporated to about 150 ml and 100 ml of water was added. The sulfuric acid was removed by passage of the solution through a column of ion exchange resin IR45 in the base form. The eluate from the column was evaporated to a small volume and freeze dried; yield 0.3 g.

#### Enzymolysis of Cane Dextran (Dextranolysis)

Cane dextran (0.3 g) containing 2.5% mannose was dissolved in 20 ml of water at pH 5.0 and treated with 5 drops of dextranase. The solution was placed in an oven for 16 hrs at 35°C. Then 1 g of filter aid and 80 ml of alcohol were added and the solution was filtered. The filtrate was concentrated and freeze dried yielding 0.2 g of dry product.

#### Thin Layer Chromatography of Alcohol Soluble Dextranolysate

The 80% alcohol soluble fraction of the dextranolysate was spotted on a 200 x 200 mm thin layer plate coated with silica gel. The plate was developed twice in the solvent, butanol, ethanol, water, acetic acid (50:30:15:5) as described by Roberts et al (1984).

The plate was dried and covered except for a 20 mm strip on either edge. The edges were sprayed with 2N sulfuric acid and the plate was heated in an oven at 105°C for 10 min to locate the separated components. The test strips indicated that 5 components were separated. The section containing each component was scraped from the un-sprayed section of the plate and hydrolyzed with 2N sulfuric acid for GC analysis.

#### GC Analysis

The GC analysis was performed with a Hewlett-Packard mode 5880A instrument fitted with a 30-m fused silica capillary column coated with DB-5, operated for 4 min at 210°C and then programmed at 4°C/min until the end of the run.

#### Gel Permeation Chromatography

A sample of the alcohol soluble dextranolysate was placed on a column of Sephacryl S-500 and eluted with water. Four fractions were collected. Each fraction was hydrolyzed for GC analysis. Detection was by refractive index (Waters Associates).

#### Dextran Produced by Organisms from Sugarcane Mud

Microorganisms were isolated from cane mud and cultured for testing on various substrates for the production of dextran as described by Jeanes (1965). The procedure is given in Appendix A.

### RESULTS AND DISCUSSION

Dextran was isolated from raw sugar from several sources. The hydrolyzed samples were analyzed by GC and contained from 1.5% to 2.7% mannose as shown in Table 1. The sample reported on in this paper contained 2.5% mannose. This particular sugar was used because it was relatively high in dextran (900 ppm) and was in ample supply.

Table 1.--Mannose in dextran isolated from raw sugars.

Sugar	% mannose
1	1.7
2	2.6
3	2.7
4	2.5

Comparison of Analytical Data

It is well known that glucose can isomerize to form mannose, especially under alkaline conditions. In order to show that the mannose in cane dextran did not arise from the isomerization of glucose, several methods of degradation were employed. Hydrolysis with sulfuric acid requires the use of barium hydroxide or barium carbonate to remove the acid and can cause temporarily basic conditions that could cause isomerization of glucose to mannose. When trifluoroacetic acid is used the acid is simply distilled off without the use of base. When acetolysis and methanolysis are used, no base is involved. The quantity of mannose found in cane dextran degraded by all of these procedures was about the same. Authentic dextran samples (Pharmacia Co.) degraded by these methods showed no mannose present.

Table 2.--Mannose in dextran degraded by different procedures.

Degradation procedure	% mannose
Hydrolysis ( $H_2SO_4$ )	2.5
Hydrolysis (TFA)	2.5
Methanolysis	0.6
Acetolysis	2.3

Enzymolysis

When the cane dextran was subjected to dextranolysis and precipitated in 80% alcohol it was found that 75% of the mannose was soluble in the 80% alcohol fraction. Thin layer chromatography of the alcohol soluble fraction was separated into 5 components which were located by guide strips along the edges of the plate. Each component was scraped from the unsprayed portion of the plate and hydrolyzed. GC analysis of the hydrolyzates showed the presence of glucose and mannose in one component. The other components showed the presence of glucose only. Therefore, at least one oligosaccharide fragment of partially hydrolyzed dextran was shown to contain mannose residues. This observation offers strong support for the presence of mannose in the dextran chain.



Gel permeation chromatography of the alcohol soluble fraction yielded 4 fractions. All fractions were hydrolyzed and analyzed by GC. All fractions showed some mannose, while fractions 2 and 4 contained most of the mannose, as shown in Table 3. Overall, the alcohol-soluble and alcohol-insoluble portions contained the same concentration of mannose, about 2.5 g in each, again indicating that mannose is part of the dextran molecule.

Table 3.--Composition of GPC separated fractions of dextran treated with dextranase.

Fraction	Composition		
	Unknown	Glucose	Mannose (I)
1	19.6	79.9	0.5
2		84.9	15.1
3	8.7	90.1	1.2
4	76.0	13.6	10.4

#### Production of Dextran from Known and Wild Organisms

Several samples of precipitated dextran from cultures of B512 Leuconostoc mesenteroides were hydrolyzed and showed no mannose present, whether the culture was grown on sucrose media or on cane juice. However, when the wild microorganisms obtained from sugarcane mud were allowed to act upon cane juice or sucrose media, some mannose was produced.

Table 4. Comparison of dextran produced by B512 and mud organisms.

<u>Culture</u>	<u>Substrate</u>	<u>Mannose (I)</u>
B512	Sucrose	0
B512	Filtered Juice	.06
Mud	Unfiltered Juice	1.2
Mud	Sucrose	2.2
Mud	Filtered Juice	0.4

The samples of dextran were all hydrolyzed by the same procedure and were analyzed by GC. From Table 4, it is apparent that wild organisms in field soil produced dextrans containing some mannose. It is proposed that these mannose residues are covalently attached to the dextran possibly as terminal groups, ensuring that no further chain lengthening occurs.



## SUMMARY

From the analytical evidence of hydrolysis procedures, acetolysis and methanolysis, it is apparent that the mannose residues observed in dextran isolated from raw sugar are not formed by isomerization of glucose during analysis.

Enzymolysis shows that mannose residues are part of the oligosaccharide breakdown products.

Synthesis of dextran from pure sucrose media or sugarcane juice-containing media, by the B512-F organism or wild strains of Leuconostoc mesenteroides show that the organisms isolated from sugarcane mud (wild cultures) produce dextran that contains mannose residues, grown on either media, while the classified organism produces, from either media, dextran containing no mannose.

It is apparent that dextran produced in sugarcane and in raw sugar contains a small fraction (2% - 3%) of mannose residues, probably covalently linked to the glucose chain. It is proposed that these mannose residues are introduced during dextran synthesis by wild strains of Leuconostoc mesenteroides found in cane field soil, and are yet to be identified.

## APPENDIX A

### Method for Production of Dextran by Leuconostoc Cultures

#### A. Culture medium for Leuconostoc mesenteroides:

- 100 g sucrose
- 2.5 g yeast extract
- 0.2 g magnesium sulfate\*7 H<sub>2</sub>O
- 5 g K<sub>2</sub>HPO<sub>4</sub> in 10 ml H<sub>2</sub>O (in a test tube with screw cap)
- Make up to 1 liter with H<sub>2</sub>O

#### B. Preparation of Leuconostoc Cultures:

Add 50 ml of the sucrose solution to a 125-ml flask and top with metal cap.

Autoclave the flask and the K<sub>2</sub>HPO<sub>4</sub> solution at 121°C for 15 min. with the cap slightly screwed on the test tube.

When the solution has cooled to room-temperature, add about 0.5 ml of K<sub>2</sub>HPO<sub>4</sub> solution to each flask with a sterile pipet and swirl gently to mix after capping the flask.

With another 1.0 ml sterile pipet, deliver 0.2 ml of sterilized water to a Leuconostoc slant. Mix the water gently over the agar surface by swirling.

Using the same pipet deliver 0.2 ml of the *Leuconostoc* solution to the flask. Swirl gently to mix.

Incubate the flasks for 4 days at 29°C. An incubator with a shaker is preferred.

C. Isolation of Dextran from the Cultures:

Place the solution in a 300 ml beaker and add 10 ml of 10% TCA. Filter the mixture through a Whatman No. 4 filter paper on a Buchner filter and save the filtrate.

Measure filtrate volume and to that add four times its volume 200-proof ethanol and 2.0 g acid-washed filter aid. Stir for 5 minutes.

Filter the mixture on a coarse, fritted glass filter and wash the precipitate four times with 80% ethanol solution. Discard the filtrate.

Boil the washed precipitate in deionized water for 25 minutes, then filter on a coarse, fritted glass filter. Rinse two times with boiling deionized water.

To the filtrate add 3 g of acid-washed filter aid, 50 ml of copper reagent (Roberts Copper Method, Clarke and Godshall, 1988).

Stir the mixture for 10 minutes and filter on a coarse, fritted glass filter. Wash the precipitate 4 times with copper wash solution.

Suspend the precipitate in 150-ml deionized water and 5 g of CG-50 resin, 100-200 mesh, and stir for 5 minutes.

Filter on a coarse, fritted glass filter and wash with 10 ml of deionized water. Discard the precipitate.

Dialyze the filtrate for 4 days; then freeze-dry.

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## DISCUSSION

Question: As you know, we in the sugar industry suffer very much when the elongated crystals come into the refinery. Will you please clarify a little further on the origins of the mannose and its effect on elongation of the crystal.

Roberts: Pure dextran alone does not seem to cause crystal elongation. However, we have not yet examined the effect of the mannose-containing dextran on crystal shape.

Question: I have a comment on the influence of dextran on crystal elongation. In our experience, we examined the influence of dextrans with different molecular weights, and there was only a trend toward elongation. So I would be very interested to know if this special dextran containing mannose gives greater elongation.

Question: The concept of a mannose on the end of a dextran chain would cause the dextran chain to be very short, probably only about 100 glucose units. Wouldn't this be too short to cause elongation?

Roberts: As I said earlier, we still do not know the effect of this type of dextran on crystal elongation. There are indications in the literature that both high and low molecular weight components are required to elongate the crystal.

We also do not yet know how the mannose is attached and are not proposing that it is at the end of every dextran chain. That will, of course, require more detailed structural studies.

Question: With regard to the elongation, we found that there is little elongation when pure dextran is crystallized with pure sucrose. However, there is no doubt that when dextran is added to a raw sugar, or when it is in a raw sugar, that dextran plus some other factor certainly causes elongation.

## STRUCTURAL STUDIES ON A FRUCTAN FROM SUGARBEET AND SUGARCANE JUICE

Margaret A. Clarke<sup>1</sup>, E.J. Roberts<sup>1</sup>, W.S.C. Tsang<sup>1</sup>, M.A. Godshall<sup>1</sup>, Y.W. Han<sup>2</sup>, L. Kenne<sup>3</sup> and B. Lindberg<sup>3</sup>

<sup>1</sup> Sugar Processing Research, Inc., New Orleans, Louisiana

<sup>2</sup> Agricultural Research Service, Southern Regional Research Center, New Orleans, Louisiana

<sup>3</sup> Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden

## INTRODUCTION

As part of an ongoing study at the Southern Regional Research Center by the Agricultural Research Service in cooperation with S.P.R.I., to identify new products that can be made from sucrose, Dr. Y.W. Han has developed a bacterial synthesis of a fructan in unusually high yield.

Fructans are polymers of fructose that occur in nature in two general forms characterized by the type of linkage between the fructose molecules (French, 1988). Inulin, the form found in Jerusalem artichokes, chicory root and many plants of the Compositae is formed by  $\beta$ -(1 $\rightarrow$ 2) linked fructose molecules and is quite insoluble in water. This type is also known as phlein or phlean. The second type, known as levans (or more properly, fructans), is formed by  $\beta$ -(2 $\rightarrow$ 6) linked fructose molecules, with some branching through the 0-1 site, is quite soluble in water. This type was thought to be a product only of microbial origin, but has been found in various grasses (Nilsson, 1988) and woody-stemmed plants in recent years. Levans are known in the beet sugar industry as microbial products that indicate sucrose loss and filtration problems in the beet factory. They are associated with deteriorated beet and with frozen and thawed beet. Structures of the two types are shown in Figure 1.

Several Gram-positive (Bacillus and Streptococcus) and Gram-negative (Pseudomonas, Xanthomonas, Enterobacter and Acetobacter) species of bacteria produce levansucrase, an enzyme that converts sucrose to fructan (levan) and glucose (Kenne and Lindberg, 1982).

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Use of a company or product name by the Department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.



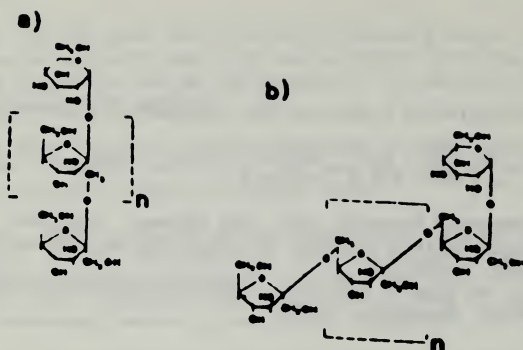


Figure 1.--Different types of fructan. a) Inulin,  $\beta$ -(1 $\rightarrow$ 2);  
b) levan,  $\beta$ -(2 $\rightarrow$ 6).

This paper will present some properties of the fructan, herein called fructan-HS to differentiate it from other fructans, and will discuss its structure.

#### MATERIALS AND METHODS

The bacterial organism was isolated from rotting sugarcane with adhering soil (Han, 1988). Strains that produced the highest yields of fructan were cultured. The cultures were grown on a sucrose medium composed of: sucrose, 7.5 g/l; peptone, 2 g/l; yeast extract, 2 g/l;  $K_2HPO_4$ , 2 g/l;  $(NH_4)_2SO_4$ , 2 g/l, and  $MgSO_4$ , 0.3 g/l. Sugarcane and sugarbeet juice, with no added nutrients, were also used as growth media. After several days, the preparation was centrifuged to remove cells, and the polysaccharide was precipitated with ethanol or isopropanol in a ratio of 60:40 alcohol:aqueous medium. The precipitate was filtered and freeze dried.

Solubility in cold and hot water was determined. Stability in acid medium was determined by monitoring an acidified solution of fructan-HS with an HPLC analysis for fructose formation.

HPLC analyses were performed on a Sugar Analyzer (Waters Associates) using deionized water as mobile phase, and an HPX-87C column (BioRad Corp.)

GLC analysis of oxime derivatives, after hydrolysis of the fructan by trifluoroacetic acid, was performed on a Hewlett Packard chromatograph model 5880, with a fused silica capillary column.

Nmr  $^{13}C$  spectroscopy was performed at 100 MHz with a JEOL GX-4000 instrument, at 70°C, with internal standard 1,4 dioxane ( $\delta$ 67.40).

Methylation analysis was run by the method of Hakomori, followed by hydrolysis with trifluoroacetic acid, sodium borohydride reduction, and acetylation. GLC was performed on a Hewlett-Packard 5970, used as an inlet for a mass spectrometer. Molecular weight was determined on a Sephacryl S-500 column (2.6 x 70 cm), using deionized water as solvent, upward flow, 2.75 ml/min, and detection by refractive index monitor, Model R-401 (Waters Associates).

X-ray crystal structure analysis was performed on a General Electric X-ray diffraction refractometer.

Optical rotation was measured on an Optical Activity Model AA-10 instrument (Tate and Lyle Process Technology, Reading, England).

## RESULTS AND DISCUSSION

The preparation and isolation of the fructan as described here provided a crude product in yields of over 80% on available fructose in sucrose. The bacterium has been identified by the American Type Culture Collection as Bacillus polymyxa (Han, 1988). This species has been reported in the literature (Evans and Hibbert, 1946). Of the many bacteria that can produce fructans (Kenne and Lindberg, 1984; Evans and Hibbert, 1946; Marshall and Weigel, 1980) the yield is generally very low, less than 10% on available fructose, or 5% on sucrose. It is thought that the fructosyltransferase (levanase) is inhibited by residual glucose from sucrose molecules. This is apparently not the situation with the species isolated for this study; hence, the yield of fructan-HS reported here in unusually high for bacterial synthesis from sucrose.

### Composition and properties

GLC analysis of the oxime derivatives of TFA-hydrolyzed fructan-HS showed over 93% fructose with a small amount of glucose and traces of degradation products. The initial molecule in fructan chain formation is sucrose; therefore, terminal glucose groups will be present in fructan-HS. Some free glucose may have been adsorbed on the crude sample analyzed because essentially no glucose was observed on methylation analysis. At the molecular weight observed (see below), there is a very small percentage of glucose units as terminal groups.

X-ray crystal structure analysis showed no crystallinity. Fructan-HS is amorphous.

A 5% aqueous solution of crude fructan-HS, after dialysis through a membrane with 12,000 daltons cut-off, gave a single, sharp clean peak just below  $2 \times 10^6$  daltons on Sephacryl S-500. It should be noted that this peak is sharper (narrower molecular weight range) than those of the commercially available dextrans used as GPC standards. The compound is stable in aqueous solution at pH 4.5 for up to 36 hours, when monitored by HPLC analysis. Fructan-HS is

readily hydrolyzed by 0.5% oxalic acid (Evans and Hibbert, 1946). It is not decomposed by amylase enzymes.

Fructan-HS has an optical rotation  $[\alpha]^{25}_D - 47.2$ . It is non-hygroscopic, unusual in view of its high solubility. Lyophilized sheets of fructan-HS have been maintained under conditions of 25-30°C and 70%-85% relative humidity for up to 6 months. The solubility of fructan-HS is very high: up to 30% in cold water, with no apparent viscosity increase. It is extremely soluble in hot water. This high solubility is characteristic of  $\beta$ -(2 $\rightarrow$ 6) linked fructans.

### Structure

The  $^{13}\text{C}$  nmr spectra, shown in Figure 2, indicates that essentially all fructose molecules in the polymers are in the same conformation. In Table 1, nmr peaks from fructan-HS are compared to peaks from known inulin ( $\beta$ -(1 $\rightarrow$ 2) linked) and bacterial levan ( $\beta$ -(2 $\rightarrow$ 6) linked). Data clearly show the fructan-HS to be of the  $\beta$ -(2 $\rightarrow$ 6) type (Barrow et al, 1984).

Table 1.---Assignment of nmr peaks.

	1	2	3	4	5	6
Inulin (1-2)	62.2	104.5	78.5	76.6	87.4	63.4
Levan (2-6)	61.4	105.1	77.5	76.6	81.3	64.6
Fructan-HS	61.4	105.0	77.8	76.4	81.1	64.2

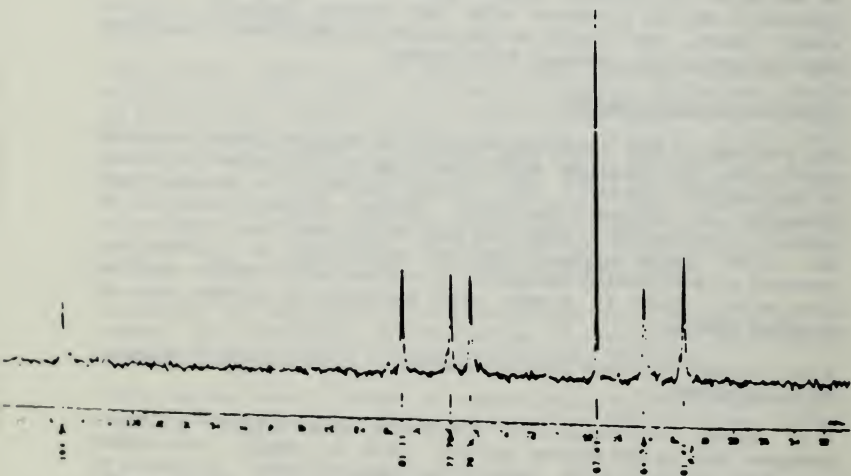


Figure 2.  $^{13}\text{C}$  nmr spectrum of fructan-HS.

After methylation, analysis by GLC provided chromatograms shown in Figure 3a, with the area of methylated monomer peaks expanded and shown in Figure 3b.

Mass spectrometric data confirm the assignments indicated on Figure 3b, and, in combination with the GLC data indicate about 12% branching in fructan-HS, as outlined in Table 2.

Table 2.--Linkages as indicated by methylation analysis.

$\beta$ -(2 $\rightarrow$ 6) linked fructose	71%
Branch points (at 1,2,6)	12%
Terminal groups (1 or 2 position)	13%
Undissolved material	4%

Branch points are indicated by the presence of 3,4 dimethyl substituted fructose, and the degree of branching of 12% is supported by the observation of 13% terminal groups, indicated by tetramethylated fructose residues, substituted at the 1- or 2-positions. The branches are formed by  $\beta$ -(1 $\rightarrow$ 2) linkages with side-chains of  $\beta$ -(2 $\rightarrow$ 6) linked residues. The degree of branching in fructans has been shown to range from 5-20% (Lindberg et al., 1973). The free hexose probably results from material that was not dissolved during methylation.

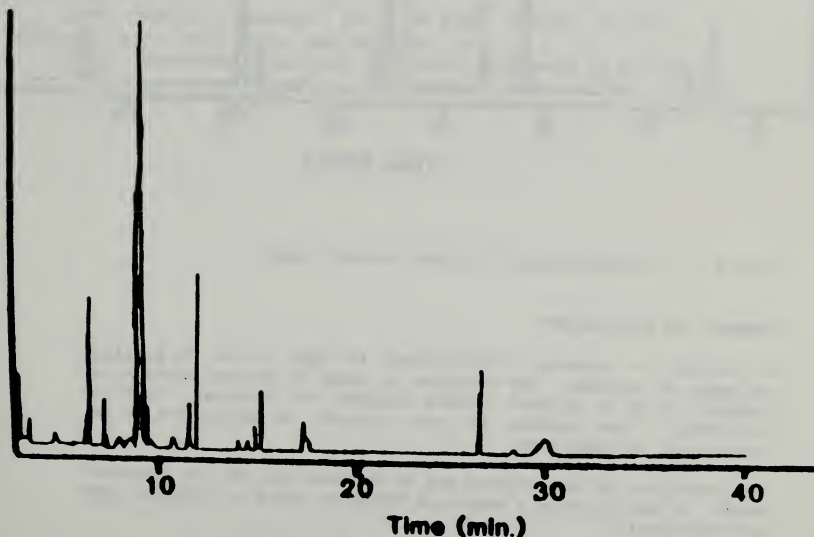


Figure 3a. GLC of methylated fructan-HS.

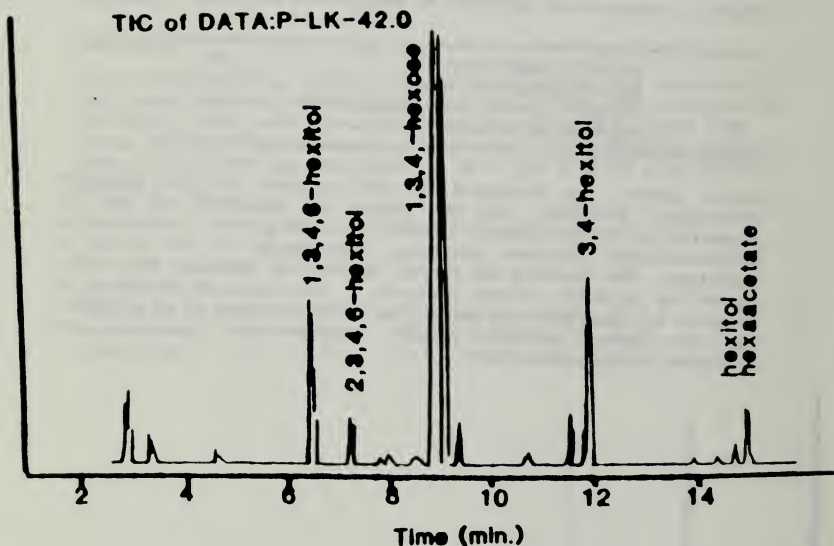


Figure 3b. Identification of alditol acetate peaks.

#### SUMMARY AND CONCLUSIONS

A fructan is reported, synthesized in high yield by Bacillus polymyxa on sucrose. The structure is shown by nmr and methylation analysis to be a  $\beta$ -(2 $\rightarrow$ 6) linked polymer of fructose, with 12% branching. The fructan is very soluble in water, but is not hygroscopic in solid form.

The properties of this fructan are of interest for use in processed foods. It may serve as a source of fructose, which is released upon acid hydrolysis.



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## DISCUSSION

Question: I think this compound could be of great interest and potential use to the industry. Have you tasted it?

Clarke: Yes, it is very slightly sweet, but not enough to use as a sweetener.

Question: I have two questions. First, what happens to the glucose moiety of the sucrose? Second, what is the molecular weight of the fructan?

Clarke: The greater part of the glucose remains in solution, as glucose, we think. There is HPLC evidence of that. The molecular weight is just under two million. The molecular weight range is very narrow, and the compound gives a very sharp peak on GPC-- sharper than the blue dextran standard marker.

## RECENT EXPERIENCES WITH A NEW FOAM OIL FOR THE CONTROL OF FOAM IN A SUGARBEET FACTORY

Simon M. Radford and Jerry R. Elvin

Tate and Lyle Process Technology, Bromley, England

### INTRODUCTION

One of the major operational difficulties of producing sugar from sugarbeet, is the occurrence of large quantities of foam. It occurs in all places during the processing of beet, from the transport water (or flume water) through to the white sugar pans. However, the most serious point of occurrence to the sugarbeet producer is in the diffuser. If foam is formed here, the sliced beet, or cossettes, will stick together causing the diffuser to plug, resulting in channelling within the diffuser and overflow of juice. It then imposes a reduction in the slicing rate to clear the plug, and a loss in the sugar extracted from the cossettes.

Along with the production problems, excessive foaming also results in bad housekeeping. It is potentially hazardous to allow large quantities of slippery foam to pour onto a factory floor. Clearly the production of foam must be controlled to prevent such problems, and this is achieved by the continual minimal addition of chemical antifoams. Originally these consisted mainly of diesel oil, palm oils or similar products. However, with the production and development of polymers, the nature of these products has changed and this plus the development of improved formulations, have resulted in improved control of foam. However, to date no one has completely eliminated foam in sugarbeet factories.

In this paper we describe the theories behind the formation and destruction of foam and recount our recent experiences in the control of foam.

### GENERAL THEORETICAL PRINCIPLES OF FOAM FORMATION

Before trying to understand the complex theory behind the production and destruction of the foam occurring in the sugarbeet process, it is important to clarify a few basic definitions (Kulcheris, 1987; Perry & Chilton, 1983).

Foam is an aggregation of bubbles separated by thin films. It consists of a gas dispersed in a liquid in a ratio such that the mixture's bulk density approaches that of a gas rather than a liquid.

Surface tension is a downward attractive force that pulls the surface molecules inward. Since molecules in the bulk of a liquid are completely surrounded by other molecules, the molecular attractions are evenly distributed and symmetrical in all directions. However, molecules at the surface are only partially surrounded by other molecules, i.e. those below the surface (See Figure 1). Therefore, the surface molecules are being pulled more firmly to the body of the liquid resulting in a molecular cohesion and orientation resembling the appearance of an invisible elastic membrane. This phenomenon is called surface tension and is responsible for a liquid's resistance to surface penetration (Berger 1975/76).

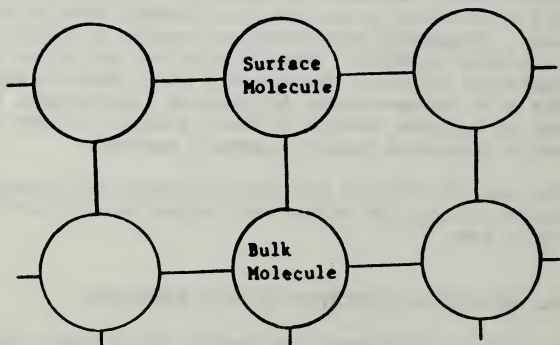


Figure 1.--The interaction of surface and bulk molecules.

Free surface energy is the differential between the energy of the molecules at the centre of the liquid, and the energy of those at the surface.

Surface activity is a change in the surface energy and surface tension of the liquid. This is caused by either changes in the physical and chemical conditions or by the addition of a surface active agent (surfactant) into the liquid. A surface energy change may be an increase or decrease in the surface tension of the liquid. Depending on the net value of this increase or decrease, and the presence of a gas in the liquid, foaming or defoaming may take place. The phenomena of foaming and defoaming are indeed one and the same--involving a change in surface tension. A decrease in surface tension results in foaming, an increase in defoaming.

Surface active agents (surfactants) change the surface tension of the liquid with which they are mixed. Generally a surfactant consists of two radicals--one which remains in the liquid (hydrophilic) and one which remains out of the liquid (hydrophobic).

Defoamers are commercially formulated surfactants added to expedite the destruction of existing foam.

Antifoams are commercially formulated surfactants added to potential foaming materials, in order to prevent foam from foaming.

#### The Mechanism of Foam Production/Destruction

Pure liquids, and saturated solutions exhibit very little foaming--it usually occurs to solutions of moderate concentration with the presence of a "foreign compound". Such a compound acts as a surfactant to reduce the surface tension and creates a surface layer composition different to that of the bulk of the liquid. In fact, Gibbs equation can predict the foam behavior of a solution.

$$Q = - (C/RT) (dY/dc)$$

Q = Excess of solute in surface over that in the bulk liquid

C = Solute concentration

R = Universal gas constant

T = Absolute temperature of solution

Y = Surface tension of the solution

With a defoamer type surfactant, the rate of change of surface tension with concentration (dY/dc) is positive and will therefore lead to foam inhibition. In contrast, with a surface active solute, the rate of change of surface tension with concentration is negative, and therefore, foaming occurs.



On the basis of this theory, it would seem relatively simple to utilise the most effective surfactant for the foam produced in the sugarbeet process. However, a reduction in the surface tension alone is not enough to reduce the foam. In fact, as can be seen from the many papers written on the subject (Berger 1974; Dedec and Novacek, 1928; Langmuir, 1924; Ewers and Sutherland, 1952; and Ross, 1971), the mechanisms for foam stabilisation are extremely complex with several factors coming into play at the same time. For example, Berger (1974) found that foam produced from diffusion juice is stabilised by 5 factors:

- 1) High surface viscosity
- 2) High surface charge
- 3) Condensed surface layers
- 4) High surface transport
- 5) Slow rate of surface absorptions from bulk solution

Clearly, the mechanism for foam production and stabilisation is extremely complex with so many factors coming into play. It is impossible to say how an antifoam exactly works without carrying out a large number of physical measurements on the foam. Choosing an effective antifoam for a particular foam from the numerous commercially available is extremely difficult. A review of chemical antifoam agents (Currie, 1953) states, "In beet sugar refining, the beets will vary in types and concentrations of foam-producing impurities. Each day or sometimes every few hours the processor may find that he must destroy foam different in chemical and physical properties. When the problem of inhibiting foaming in one mill is multiplied by the number of mills processing beets, and using different equipment, raw material, and techniques of operation, the reader will appreciate the variables which may exist among foams of the same type."

Therefore, the only way to find the best commercial antifoam for the factory is to conduct a full scale plant trial.

#### Foam Destruction and Inhibition

Foam destruction, or defoaming, should be distinguished from foam inhibition. Defoaming can be performed by physical, mechanical, or chemical means, but foam destruction does not necessarily prevent the liquid from foaming again. Foam inhibition employs a chemical foam inhibitor that not only destroys the foam but also prevents additional foaming (antifoam).

### Physical and Mechanical Defoaming

The destruction of foam by physical or mechanical means involves applying a shock to the surface of the foam to break it down by collapsing the bubble film. There are a range of different devices for achieving this--the shock can be physical, thermal, electrical, mechanical or acoustical and can be applied by a variety of devices.

Such devices include static or rotating breaker bars, headers that shower the foam with a fine spray of liquid or steam, high frequency air pulses, or contacting the foam with a hot surface. In all these cases, the foam is broken down by the sudden change in its elasticity. However, in general, these devices have low efficiencies and their effectiveness is unpredictable.

### Chemical Defoamers/Antifoams

In the case of chemical defoaming, foam destruction and formation may be in equilibrium, and is determined by the quantity of antifoam used and the physical and chemical conditions. However, in addition to knockdown ability, the chemical must sustain foamless conditions for a prolonged time. An effective antifoam should destroy the foam at its source. It should also inhibit foam formation if the conditions that produce foam persist.

Chemical antifoams have the disadvantage that they constitute the addition of a contaminant to a process stream. For the case of sugarbeet processing they are often inert, and used in such small quantities that there is no harm done to the final product.

### Desirable Antifoam Characteristics

Most of the commercially available antifoams are proprietary formulas to prevent copying of an effective product by a rival company. However, there are some general characteristics that all the commercially acceptable antifoams conform to.

In order to be used in a factory producing sugar, the constituents of the antifoam must conform to any Federal or State safety regulations as well as subject to the limitations imposed by the U.S. Food and Drug Administration.

Most of the commercially used antifoams are liquids--some are soluble in water and others are not. The dispersible products are often preferred in applications where the antifoam is supplied as a concentrate to reduce its transport cost.

Other physical properties that need considering are viscosity, pour point, flash point, clarity, and corrosiveness. The requirements for each of these properties will vary from factory to factory depending on climate, dispensing, handling, and storage facilities.

Ideally, the product should be designed to have excellent foam destroying properties, prevent foam from appearing, and be capable of carrying these properties as far as possible down the process. Some manufacturers separate their products into defoamers (good destroying properties) and antifoams (good prevention properties), but it is possible to combine both properties in one product.

Since antifoams are surface active compounds, care must be taken that they do not collect on surfaces where they will create other processing difficulties. An example would be evaporator tubes where they may retard rates of evaporation. Antifoams are designed to be used over a specific concentration range and using too much may actually enhance foam (Ross and Young, 1951).

Finally, it is essential that the antifoams are dosed correctly. In our experience, improved dosing alone has led to better control of foam and a subsequent saving of factory money.

#### The Talox A Antifoam

As previously mentioned, due to the highly complex mechanisms involved in the production of foam, and the large number of variables in the sugarbeet process, the best approach to pinpointing the most effective antifoam is to conduct a plant trial. The most important foam to control in the factory is that produced in the diffuser. If the juice is foaming, there could be a loss of up to 1% sugar to the pulp. The use of an antifoam reduces this to 0.15% (Berger, 1974) with a consequent saving of money.

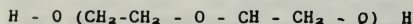
Initially, in order to choose the most effective antifoam for the diffuser, three different products were developed for use on a plant scale:

Talox A            A high temperature antifoam.

Talox C            Effective between 10 and 90°C.

Talox D            Effective over a broad temperature range.

All these products consist of a formulation based on a block copolymer of Ethylene Oxide and Propylene Oxide:



Talox range contains all or some of the following:

Dimethyl polysiloxane  
Polyethylene glycol  
Polyethylene glycol esters of tall oil fatty acid  
Mineral oil  
Polypropylene glycol  
Polypropylene glycol esters of tall oil fatty acid  
Polyethylene - polypropylene glycol copolymers  
Polyethylene - polypropylene glycol copolymers  
    esterified with tall oil fatty acid  
Tall oil fatty acid  
Soya oil  
Soya fatty acid  
Isopropyl alcohol  
Petroleum wax  
Butyl alcohol  
Castor oil  
Cyclohexanol

The exact mixture and proportions are, of course, a proprietary secret. However, they differ in proportioning, minority constituents and manufacturing techniques.

Despite the differences, the main aims of the product must be:

1. Eliminate the foam
2. Inhibit foam formation
3. Inhibit absorption on organic matter.

All the products were dosed at the points shown in Figure 2.

Of the three test products it was soon clear that the Talox A product was the most effective. It excelled at destroying existing foam in the process, had an excellent inhibiting effect and had reasonable "carry through" properties. In fact, the Talox A was reduced to less than half of the normal antifoam consumption, and the diffuser ran well with no foam problems. However, during these first trials the problems with dosing antifoams were realised, and it was thought that improved dosing alone could lead to a reduction in foam oil use. The problems of dosing the products effectively were similar in the other sugarbeet factories. When you are using a cheap product the control of the product is often poor and more product than necessary is used. Anybody who has worked for any length of time in the beet industry has heard of the "bucket brigade". Where foam appears throw a bucket of foam oil at it.



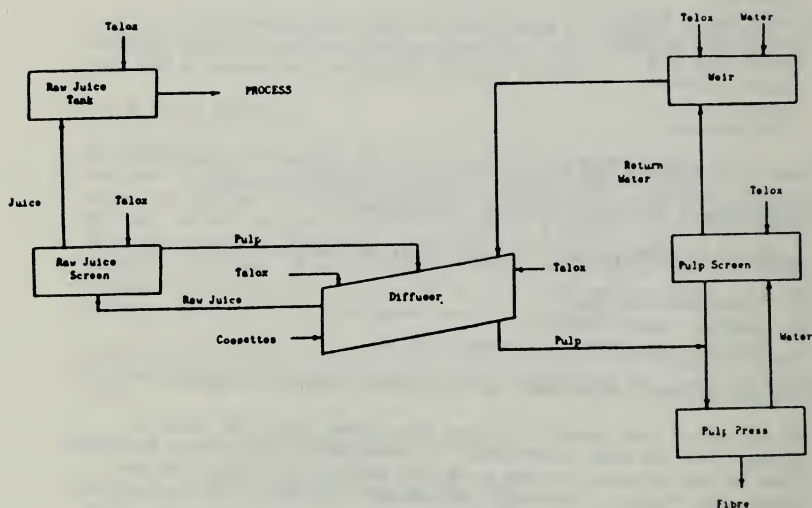


Figure 2.--The dosing point of the antifoam.

However, with the introduction of a premium product and the recent escalating costs of the raw materials, coupled with a highly competitive market, it was economically essential not to waste products by transporting antifoams through poor dosing systems. In order to ensure a fair and scientific trial, with antifoam being dosed in the required part of the process, the Talox Antifoam Dosing System (TADS) was developed. This was specifically designated to give maximum control and effectiveness of antifoam dosing. The TADS was designed as two ready assembled units mounted in portable weatherproof cabinets.



Having developed this extremely reliable system as well as establishing that Talox A was the most effective of the test foam oils, a foam oil trial was set up in four sugarbeet factories. It was only at this point that a comparison could be made between the Talox A product and its rivals. The work was performed at the start of the 1986-87 campaign. Figure 3 shows the process flow diagram for the TADS system.

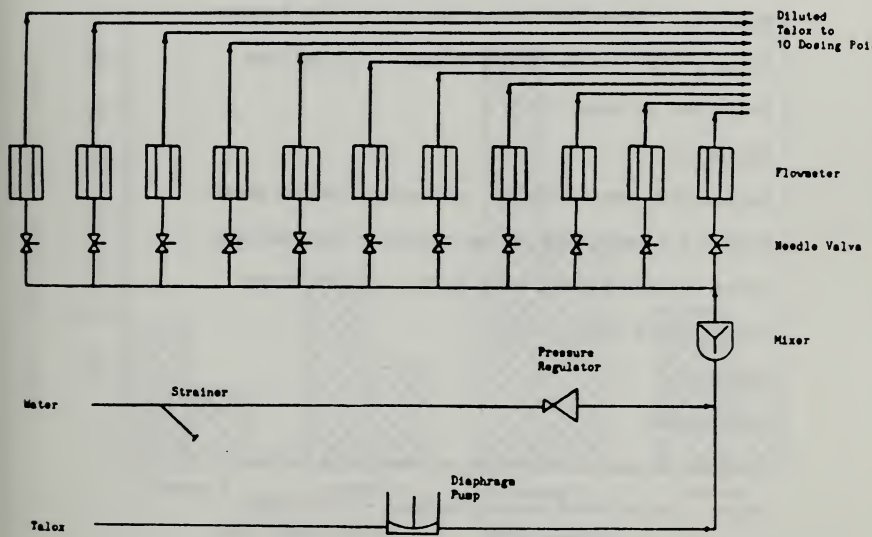


Figure 3.--Process flow diagram of the Talox antifoam dosing system (TADS).

## TRIAL RESULTS

### Factory 1

The dosing system was installed alongside the system already being used. Talox A was turned on with the following results:

#### Consumption

Foam Oil X (with old dosing system) = 28.5 Gal/day

Talox A (with Talox Dosing System) = 15.5 Gal/day

Reduction in usage = 46%

### Factory 2

Talox A with the TADS was turned on in the main process, while foam oil X was continued to be dosed in the Steffen House.

#### Consumption (Main Process, excluding Steffen House)

Foam Oil X (with old dosing system) = 37.8 Gal/day

Talox A (with Talox Dosing System) = 20 Gal/day

Reduction in usage = 47%

### Factory 3

#### Consumption (Main Process, excluding Steffen House)

Foam Oil X (with old dosing system) = 82.4 Gal/day

Talox A (with Talox Dosing System) = 46 Gal/day

Reduction in usage = 44%

### Factory 4

#### Consumption

Foam Oil X (with old dosing system) = 32 Gal/day

Talox A (with Talox Dosing System) = 20 Gal/day

Reduction in usage = 38%

Figure 4 compares the consumption of Talox A to the previously used foam oil.

Since Talox A is a premium foam oil it is more expensive than most of the foam oils on the market (Figure 5). However, due to the dramatic reductions in consumption, a change to Talox A still meant a large reduction of the foam oil cost.

Since this campaign, these factories have been using Talox A antifoam.

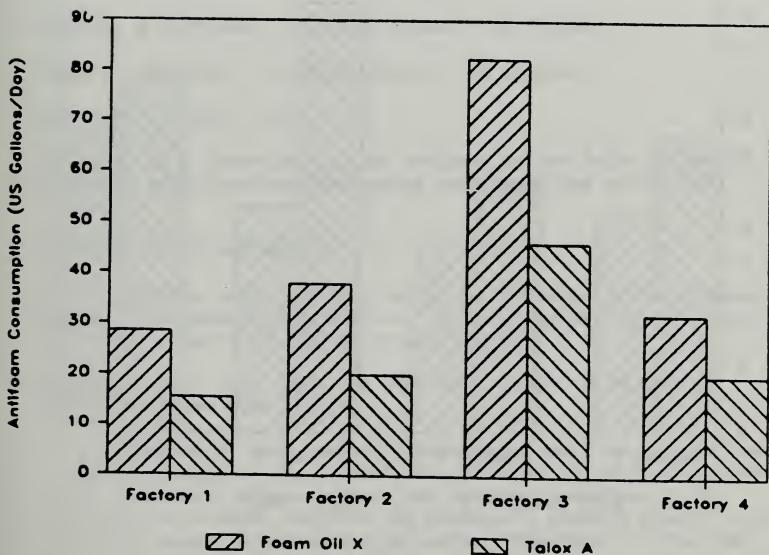


Figure 4.--The consumption of Talox A against the foam oil previously used at the factory.

# THE TALOX DS ANTIFOAM

Having done this successful work in these four factories, it was clear that although Talox A excelled in the warm temperatures of the diffuser, it had very little effect in the Steffens process. The Steffens process is a method by which sugar is recovered from beet molasses. Due to the recycling nature of the process a factory can only recover sugar from molasses supplied by other factories. Their own molasses are sold as normal.

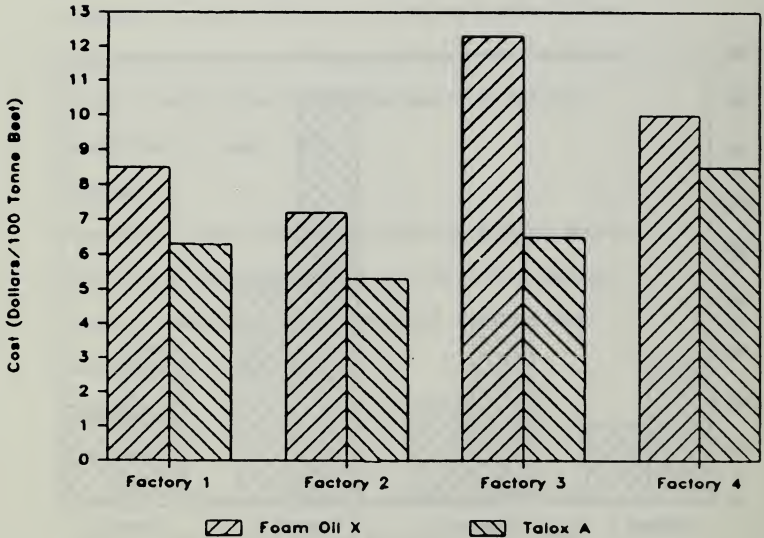


Figure 5.--The cost of Talox A against the foam oil previously used at the factory.

The process works by adding lime to diluted molasses and cooling to approximately 14°C. Calcium sucate is precipitated out, removed on rotary vacuum filters and returned to the process. The liquor is heated where further calcium sucate is precipitated and this is returned to the molasses dilution tank.

If foaming occurs in this process, temperatures in the first step are increased to reduce the foam (the antifoams are generally more effective at high temperatures), pick up on the R.V.F. is less efficient and recovery is reduced. And, of course, foam fills the factory. With both high and low temperatures it is essential that the temperature range of the antifoam is broad (10-90°C).

For this market, a product known as Talox DS was specifically formulated and tested during the 1987-88 campaign. This was dosed in the six points around the Diffuser (see Figure 2) and four points in the cold Steffens process (Figure 6).

### Trial Results

Consumption:

Foam Oil X (Diffuser + Steffens House) = 40 Gal/day

Talox DS (Diffuser + Steffens House) = 36 Gal/day

Reduction in usage = 10%

Not only was a reduction in cost achieved by the Talox DS but it gave more effective foam destroying and inhibiting properties.

### FUTURE PRODUCTS AND MARKETS

In the next few years, we hope to extend the usage of our premium foam oil throughout North America. However, we are not oblivious to the highly competitive market we are entering.

We also believe that there are big improvements to be made in quality of flume foam oils.

It is also thought that further improvements could be made in the dosing system--it has been found that dilution of foam oil has very little beneficial effect. Since the dilution water has to be evaporated at a later stage in the process it means a greater steam usage, and hence larger operation costs. Therefore, if the foam oil can be applied directly without increasing its consumption, this could be of economic benefit to the factory.

It may also be worthwhile considering the automatic control of the antifoam. Foam behavior is extremely unpredictable and it would be economically advantageous if antifoam could be dosed at the points of detection of this foam.



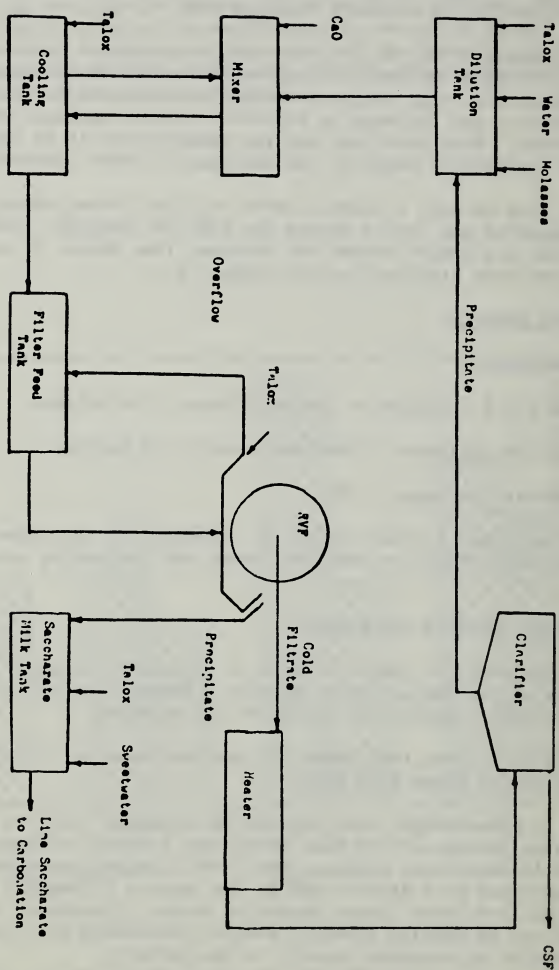


Figure 6. Dosing points of Talox DS in the Steffen House.

We believe that premium products are more cost effective to the sugarbeet industry and will work to ensure their wide use in the sugar industry.

Finally we would like to thank all the people at Western Sugar who let us fill their factories with foam and were very patient until we got things right.

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#### DISCUSSION

(The paper and discussion were presented by J.R. Elvin.)

Question: Some years ago, we had a research project on antifoaming agents. At that time, we reacted sucrose with ethylene oxide or propylene oxide and tall oil, so we had something similar to your product. The product was a mixture that required fractionating to make a more effective anti-foam. I wonder if you fractionated your product?

Elvin: No, we did not. We used the mixture.

Question: Over what period of time were the results obtained showing the cost-effectiveness of the product versus conventional antifoam products?

Elvin: There are two factors that determine the efficiency of an antifoam. One is: whether it does what it's meant to do--destroy foam. That you know very quickly. If a new product does not work, you can tell within an hour.

The second: the reduction of costs, must be done over a campaign. We tested this product over a campaign. It is always very difficult to compare one to one, because of other varying factors. The only comparison we have is over one campaign. At some point then, the factories reverted to the previously used product, and consumption and costs increased.

Question: Foaming is often a problem in cane sugar factories using carbonatation and in refineries. Have you looked at the application of your compounds to these plants?

Elvin: No, not yet. We've been involved with beet factories only.

# THE APPLICATION OF FLOTATION CLARIFICATION IN SUGAR PROCESSING

P W Rein

Tongaat-Hulett Sugar, South Africa

## INTRODUCTION

Flotation clarification is a process which has found particular use in water treatment and in the mineral processing industry. The process has also been used for many years in sugar refining in the form of phosphatation.

Clarification by gravity settling of syrup and other high brix materials encountered in sugar processing is not possible because of the high density and viscosity of the syrup. As an alternative to filtration, which is an expensive unit operation, flotation clarification can be considered as a simpler and cheaper option.

In this process, very fine suspended matter can be floated to the surface by fine air bubbles. Separation by flotation does not depend so much on size and relative density of particles being removed as it does on their surface properties. The process can be considerably enhanced through the use of appropriate chemicals. In the mining industry selective flotation of particular types of particles is possible through the addition of chemicals which change the surface properties. The use of flocculants in flotation also leads to mechanical entrapment of air bubbles in the floc particles.

Application of flotation clarification in raw sugar mills was pioneered by Tate and Lyle (Bennett et al 1977) and a number of installations clarifying syrup have been installed throughout the world. Other systems have been installed in Hawaii, Taiwan and South Africa.

The development of syrup clarification and its application in Tongaat-Hulett Sugar has been reported elsewhere (Rein et al 1987). The application of syrup clarification followed an extensive laboratory investigation which showed the advantages of applying flotation to raw syrup. The laboratory investigations also covered the application of flotation clarification to a number of other streams in raw sugar mills and in cane sugar refineries. Details of the laboratory work undertaken are given in this paper, together with an outline of experience gained with three syrup clarifiers in Southern Africa.

## RESULTS OF LABORATORY WORK

The mechanisms involved in the formation and flotation of floc particles are extremely complicated. The approach adopted in this investigation was not to try to understand the mechanisms involved but to undertake a series of empirical investigations to establish where the process may be viable. An important part of the investigation therefore was the development of a suitable laboratory test to simulate the process in practice.

### Details of Laboratory Equipment

A laboratory test was devised which involved rapid stirring of syrup for three minutes in a small beaker held in a water bath at constant temperature. The stirring arrangement was such that air was entrained into the liquid and dispersed by the impeller to saturate it with air. Polyelectrolyte was gently stirred into the contents of the beaker for 30 seconds after aeration and the liquid was left standing for twenty minutes. After this time the separation between scum and clear liquid was complete and samples of clear liquid could be taken off for analysis. Milk of lime and phosphoric acid, when used, were added to the liquid before aeration.

In some cases, solutions were heated to 75°C, limed and then gassed down with either CO<sub>2</sub> or SO<sub>2</sub> as quickly as possible to the required pH level. These processes were referred to as carb-flotation or sulflotation respectively. Thereafter flotation was carried out using the standard procedure of aeration for three minutes followed by flocculant addition.

An important analysis in evaluating flotation clarification is the measurement of turbidity. The method in the laboratory manual for South African sugar factories (SASTA 1985) was found to be unacceptably sensitive to small changes in pH. Therefore a revised procedure was used, details of which are given elsewhere (Rein et al 1987). This involves measuring absorbance at 720 nm.

Colour was measured using a standard method based on the ICUMSA procedure (at 420 nm) and standard procedures for ash and gums were used (SASTA 1985).

### Method of Aeration

Experience in sugar refining (Saranin 1972) and water treatment has indicated that dissolved air flotation gives better results than the simpler aeration used in these tests, which would be classed as dispersed air flotation. Thus a laboratory system was devised to investigate dissolved air flotation by saturating the liquid at 500 kPa for five minutes before clarification. Release of pressure results in gas coming out of solution, forming large numbers of small bubbles. Under the microscope,



it was evident that dissolved air flotation gave a more uniform bubble size of about 100  $\mu\text{m}$ . With dispersed air flotation, the bubble size was larger and much more variable. However, laboratory trials indicated that dissolved air flotation showed no advantage, and if anything, gave worse performance. In the interests of simplicity and cost, dispersed air flotation was adopted.

### Syrup Clarification

The bulk of the work done has involved clarification of syrup from a raw sugar mill. This has a brix of between 65 and 70 and a purity of 85 on average. It was found with this material that aeration led to a very stable scum layer and that a high turbidity removal could be achieved merely with the addition of poly-electrolyte.

Microscopic examination showed that the majority of the particles in unclarified syrup were in the range 1 - 10  $\mu\text{m}$ . In the scum, it was evident that floc sizes were very much larger, surrounding the air bubbles.

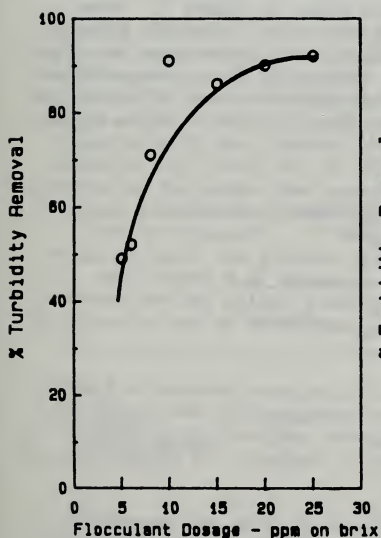


Figure 1.--Turbidity removal in syrup as a function of flocculant dosage

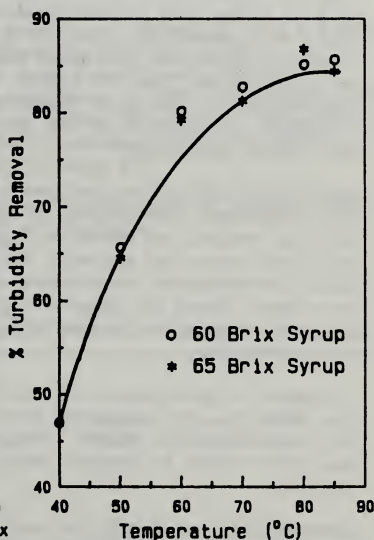


Figure 2.--Effect of temperature on turbidity removal

Laboratory trials showed that there was no significant purity improvement and that the amount of ash removed was small. In addition the percentage improvement in syrup colour was small, perhaps because of the high syrup colour. However the removal of turbidity was dramatic.

The effect of flocculant dosage on turbidity removal is shown in Figure 1. This indicated that a dosage of around 15 ppm on brix was optimum. Subsequently it has been found that with very poor quality syrups, higher dosage rates of up to 30 ppm are required for best results. It has also been established that Talosep A3 and Separan AP273 gave better results than Magnafloc LT27. In general it has been found that polyelectrolytes with a higher degree of hydrolysis and lower molecular weight do give better results in this application.

Temperature was found to have a significant effect on turbidity removal. This is shown in Figure 2, which indicates that a progressive improvement is obtained up to 85°C, after which the effect of temperature levels off.

From Figure 2 it can also be inferred that turbidity removal is independent of the brix and viscosity of the syrup. The viscosity of the 65 brix syrup is about 50% higher than that of the 60 brix syrup used in the results shown in Figure 2. Other tests also showed no effect of syrup brix. Clearly, therefore, viscosity values in the range encountered do not have an effect on this process, and the better results at higher temperatures are not due to viscosity reduction but due to the effect on the formation of flocs or on the intramolecular attractions across air/liquid interfaces.

Both clarified and unclarified syrup were subjected to laboratory boilings. Viscosities of molasses produced were measured and the results are shown in Figure 3. Viscosity of the clarified syrup and molasses after boiling was lower by up to 25%. A reduction in molasses viscosity is a significant advantage, since the viscosity affects curing and the ultimate molasses exhaustion is generally controlled by the viscosity of the masseccuite.

Attempts to investigate the effect on sugar quality of clarifying syrup in the laboratory were unsuccessful, due to the lack of reproducibility of the laboratory pan boiling procedure. It was left to the full-scale tests to evaluate the effect on sugar quality.

Generally it was found that addition of lime and phosphate, particularly at higher lime levels, did not improve the results compared to the standard process adding only flocculant. Since phosphoric acid is very expensive, this is a welcome result.

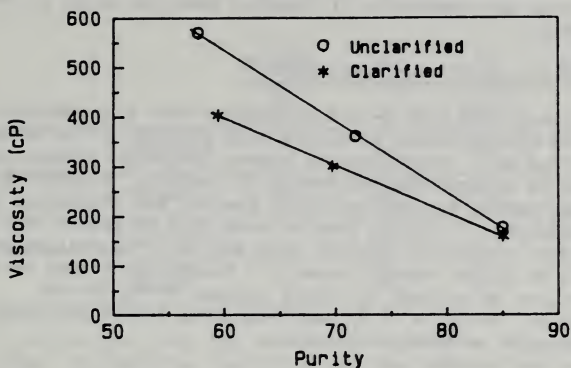


Figure 3.--Viscosity of syrup and molasses (adjusted to 73 brix and 40°C) with and without syrup clarification

Gassing with CO<sub>2</sub> proved also to have no advantage, but a significant improvement could be obtained with SO<sub>2</sub> gassing, i.e. sulflotation. In this case, best results were obtained by adding 0.3% lime and 300 ppm phosphate on brix and gassing with SO<sub>2</sub> to a pH between 6.0 and 6.5 before flotation. Results are compared with the standard clarification process in Table 1, using 65 brix syrup and 20 ppm Talosep A3.

Table 1.--Comparison of standard syrup clarification with sulflotation

	Turbidity	% Removal	Colour (420 nm)	% Removal	Ash (%)	% Removal
<u>Run 1</u>						
Untreated	1.006	-	29100	-	2.84	-
Standard process	0.119	88.2	27600	5.2	2.45	13.7
Sulflotation	0.042	95.8	23100	20.6	2.42	14.8
<u>Run 2</u>						
Untreated	0.992	-	28000	-	2.80	-
Standard process	0.107	89.2	26000	7.1	2.55	8.9
Sulflotation	0.051	94.9	21000	25.0	2.64	5.7

Clearly sulflotation gives significantly improved results, and in particular, a useful colour reduction. However, the additional cost and complexity of chemical addition needs to be considered.

#### Clarification of B- and C-molasses

It was found that both B- and C-molasses were also amenable to flotation clarification providing some dilution of the molasses was carried out. Significantly higher flocculant dosages were required to achieve maximum turbidity removal. Figure 4 gives the relationship between turbidity removal and flocculant dosage for B-molasses. A dosage rate of more than 50 ppm on brix gave a turbidity removal of greater than 80% but a removal of 90% was possible at higher dosage rates. A flocculant dosage rate of 160 ppm gave an average turbidity removal of 90.7% with C-molasses. Analysis of B- and C-molasses before and after clarification is shown in Table 2.

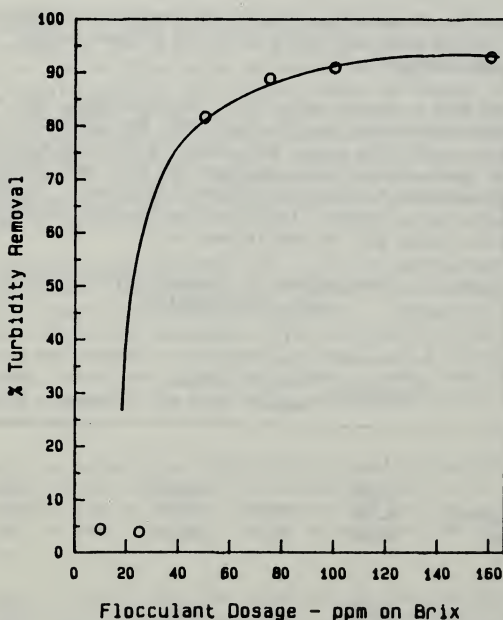


Figure 4.--Turbidity removal in B-molasses as a function of flocculant dosage

Table 2.--Analysis of B- and C-molasses before and after treatment by clarification

		Turbidity Removal (%)	% Solids	GC Sucrose Purity	Monosaccharide/ Ash
B-molasses	Untreated		55.7	50.9	0.62
	Treated	88	54.5	52.8	0.72
C-molasses	Untreated		52.3	42.9	0.50
	Treated	91	50.9	44.9	0.57

It can be seen that the molasses purity rises by about two units. In addition removal of ash was achieved which can be seen in the higher monosaccharide/ash ratios of the treated molasses. This should improve molasses exhaustion slightly. Boiling down tests undertaken in the laboratory on clarified and unclarified C-molasses otherwise found no change in the final equilibrium purity. However a viscosity reduction of 24% between untreated and treated final molasses indicated that C-masseccutes would be less viscous, and in practice therefore some reduction in final molasses purity may be achieved.

B- and C-molasses were the only materials tested which showed an effect of brix on turbidity removal, perhaps because of the very high viscosity levels. The molasses generally had to be diluted to give a viscosity below 100 cP to achieve good results, but this limit was not well established. Total solids contents greater than 50% led to lower turbidity removals.

In general it was found that the quantity of organic non-sugars was higher than the quantity of ash in the scum.

De-sweetening of the scum from C-molasses clarification with equal weights of water and scum resulted in 70% of the sucrose being recovered and 20% of the ash in the scum being re-dissolved. Where there is a need to recover all the sucrose in the scum, as in B-molasses clarification, up to 50% of the ash can re-dissolve.

#### Filtrate Clarification

Filtrate from rotary vacuum filters is normally recycled because its quality is inferior to that of clarified juice. Samples of filtrate were subjected to flotation clarification to assess whether the quality can be improved by this technique. Tests were undertaken at normal filtrate temperature ( $\pm 65^{\circ}\text{C}$ ).



Initially it was found that the suspended solids had a tendency to settle rather than float. However it was established that if the aeration rate could be increased (in this case by using a more powerful stirrer) and if the flocculant dosage rate was kept above 20 ppm Talosep A3 on brix, roughly 85% of the turbidity could be removed. If Separan AP 273 was used, more than 50 ppm flocculant was required. These results are in accord with other work indicating that high flocculant quantities are necessary for filtrate clarification (Tong et al 1986).

In this case, the addition of 300 ppm phosphate and lime increased turbidity removal to a point where the clarified filtrate was of better quality than the clear juice. The use of cationic flocculants appeared to give a slight colour improvement of 10 - 20%.

#### Melt Clarification

The possibility of applying this process to melted raw sugar was investigated. This was done particularly for Triangle sugar mill in Zimbabwe who were required to make white sugar by remelting raw sugar and re-crystallising.

The standard process applied to clarification of melt adding only polyelectrolyte to the melt gave a fairly low turbidity removal, of the order of 25%. However the turbidity level in the melt is only about one-tenth of that in sugar mill syrup. The lower turbidity removal on melts was confirmed when tests were done on remelt of B- and C-sugars in the raw sugar mill. There too, turbidity removals in percentage terms were significantly lower.

Of interest however in clarifying melt is removal of colour and ash. In this respect a colour removal of about 20% was possible and in some cases also a significant ash removal.

Refineries using carbonatation or sulphitation obtain fairly large colour reductions on melt syrups of approximately 50%. However an expensive filtration system is needed to remove the precipitate from the syrup and lime usage is high, approximately 0,7% on solids. A series of tests was planned to see if the precipitate formed in carbonatation or sulphitation could be removed relatively inexpensively by flotation while still obtaining a useful colour removal.

Gassing with either  $\text{CO}_2$  or  $\text{SO}_2$  before flotation significantly improves the turbidity and colour removal. It was found that at lime dosages of greater than 0.3% on melt solids, performance dropped off significantly. Some results are given in Table 3, which shows results obtained on a melt made of Triangle sugar at 66 brix at a dosage rate of 20 ppm Talosep A3.

Table 3.--Comparison of carbflotation and sulflotation on melt

Sample	Turbidity	Colour (420 nm)	Ash %	pH after Liming	pH after Gassing	% Turb. Removal	% Colour Removal	% Ash Removal
Untreated	0.027	1 630	0.05	-	-	-	-	-
No lime addition	0.022	1 390	0.01	-	-	17	15	80
Carbflotation								
0.05% Lime	0.018	1 120	0.07	9.5	7.0	34	31	(+40)
0.1% "	0.031	1 120	0.08	9.8	7.0	(+17)	31	(+60)
0.2% "	0.051	1 150	0.12	10.2	6.9	(+88)	29	(+140)
Sulflotation								
0.05% Lime	0.011	1 140	0.08	9.4	7.0	59	30	(+60)
0.1% "	0.013	1 120	0.12	9.9	6.5	51	31	(+140)

Colour removals are good and appear unrelated to the quantity of lime used. Down to 0.05% lime on solids, turbidity removal was better with sulflotation and improved as the lime quantity decreased. Ash gains are evident particularly at higher lime dosage rates. Results point towards low lime dosages with either carbflotation or sulflotation.

With melt clarification the effect of polyelectrolyte appears to be different to that with syrup. Some results obtained on different polyelectrolytes at 15 ppm on solids, using a melt of Maidstone sugar at 71 brix are given in Table 4.

Table 4.--Comparison of different flocculants on carbflotation of melt

Flocculant	% Lime	Turbidity	Colour (420 nm)	% Turbidity Removal	% Colour Removal
Untreated	-	0.073	2 580	-	-
Talosep A3	0	0.063	2 060	14	20
Magnafloc LT 27	0	0.056	1 260	24	51
Talosep A3	0.1	0.237	1 160	(+224)	55
Magnafloc LT 27	0.1	0.056	1 030	24	60
Mafloc 8005	0.1	0.921	1 320	(+1160)	49
Separan AP 273	0.1	0.328	1 450	(+349)	44

Magnafloc LT 27 clearly gives the best results in terms of both colour and turbidity removal. This flocculant has the lowest anionic character of all those tested and indicates that further work using non-ionic flocculants should be considered.

From these results it was apparent that both carbflotation and sulflotation enhanced turbidity removal and colour removal from Refinery melt. This is achieved at a very low lime addition rate of less than 0.1% on melt solids.

#### Refinery Sweetwater Clarification

The sweetwater used to dissolve raw sugar often has a high colour. At Hulett's Refinery, this is derived from sweetening off carbonatation cake, and contributes to additional colour in the melt. Reduction of colour in sweetwater would therefore reduce the colour of melt. The standard laboratory test procedure was applied, using 20 ppm Talosep A3 as a flocculant. Results are given in Table 5.

Table 5.--Results of clarifying Refinery  
sweetwater

Pre-treatment	Final pH	Turbidity Removal %	Final Colour (420 nm)	Colour Removal %
None	-	56	1789	8
Lime only	8.9	84	1986	15
300 ppm phosphate and lime	8.1	100	854	43
300 ppm phosphate and lime	8.6	100	648	36
10 ppm cationic polymer (Anikem 4607)	-	46	1241	24

Turbidity removal is less important than colour removal in this instance. With the use of phosphoric acid and lime, a significant colour removal can be achieved. Optimisation of phosphoric acid dosage and use of the correct cationic flocculant could probably give better removals than those shown here.

#### EXPERIENCE WITH FULL-SCALE SYRUP CLARIFIERS

The mill at Empangeni always suffered from poor cane quality. This led to extreme difficulty in producing VHP quality sugar and to highly viscous massecuites, which meant that the exhaustion of final molasses was also poor. Syrup clarification was seen as a process which would help to overcome these difficulties, and initial work was aimed at establishing the design parameters for a new installation at this mill. The syrup clarifier operated at this mill for two years before the mill was closed down. Subsequently its cane has been processed at the new Felixton mill where a syrup clarifier was also installed. This has now been in operation for five years.

A syrup clarifier of the same design was installed at Triangle Sugar Limited in Zimbabwe at the start of the 1987 season. The process flow scheme can be illustrated by reference to the flow sheet for Triangle, as shown in Figure 5. The raw syrup is pumped through a direct contact heater where the syrup is heated up to 85°C by contact with Vapour 1, then passes through an aeration stage where air is dispersed into the liquid. This consists either of a high speed aeration pump, or a suitably designed air sparger. The liquid then flows to a small feed tank where undispersed air has a chance to disengage before the aerated syrup enters the bottom of the syrup clarifier. Just before the syrup clarifier, polyelectrolyte is added ahead of an

in-line mixer. The in-line mixer was designed to induce good dispersion of the flocculant within the liquor without significant shear which could lead to breakdown of the floc. Finally the syrup enters through the central feedpipe, the diameter of which increases as the syrup enters to reduce inlet velocity to a minimum.

The syrup clarifier itself is sized to have a retention time of around 20 to 25 minutes.

A rotating scum rake scrapes the scum off into a launder around the periphery of the clarifier and the scum flows back to the mixed juice tank. Clarified syrup is drawn off from the bottom of the clarifier through a variable height weir. The scum level in the clarifier is controlled by regulating the outlet weir in the overflow box. The clarified syrup passes to a flash vessel to evaporate the heating steam added which raises the brix to the original value and cools the syrup. Heating of the syrup at Empangeni and Felixton is done indirectly using a heater.

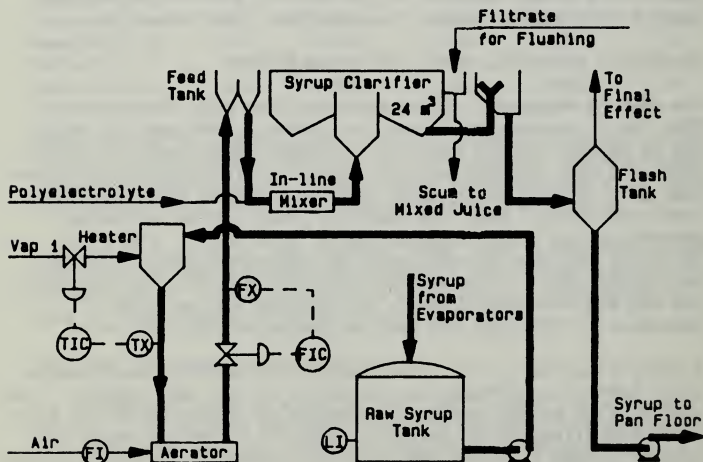


Figure 5.--Process flow diagram for Triangle syrup clarification



A diagrammatic sketch of the Triangle clarifier is shown in Figure 6.

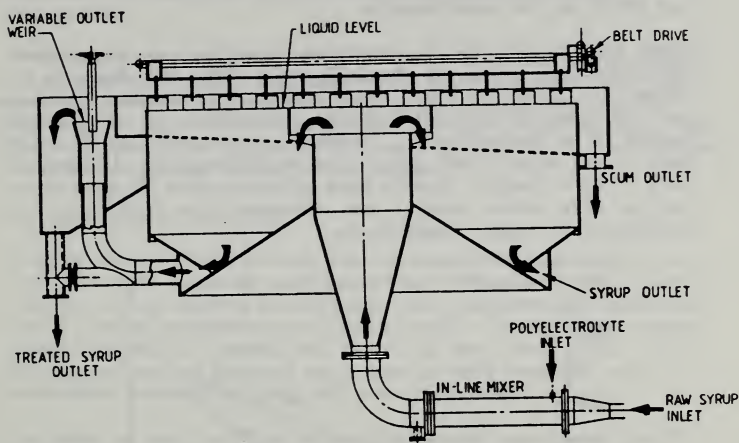


Figure 6.--Sketch of the syrup clarifier installed at Triangle

#### Empangeni Syrup Clarifier

The first season in operation at Empangeni was used to iron out design and commissioning problems and also to undertake a lengthy evaluation programme involving running the process for periods of two weeks in operation and two weeks off, in order to assess the effect on sugar quality, and boiling house performance as well. During this time, average turbidity removal was only just about 70%. Nonetheless, some significant improvements in sugar quality were achieved. A significant decrease of 15% was found in colour and gums of affinated sugar. A 15% reduction in the ash of the affinated sugar was also measured, but the difference was not established statistically. Detailed results have been previously reported (Rein et al 1987).

In practice, the effects of the syrup clarifier operation were extremely marked. Analysis of data for A-masseccuite exhaustion and the purity rise on A-curing show better results with the syrup clarifier in operation, indicating that less washing was necessary to achieve the required VHP sugar pol.

Although there were not enough data points to establish whether there was a significant effect on boiling house recovery, it was evident from the results that with the syrup clarifier in operation, the final molasses viscosity was reduced by about 25%. The mill were able to take advantage of the lower viscosities by boiling higher brix massecuites and by using less steam and water on the centrifugals.

Other results obtained in assessing the effect of temperature and flocculant dosage on the full-scale plant are shown in Figure 7. Provided the minimum quantity of air was introduced (of the order of  $0.04 \text{ m}^3/\text{m}^3$  syrup), the effect of air quantity seemed to be insignificant. However, temperature was found to have a very significant effect, as established in the laboratory. Also, better results were obtained with 30 ppm flocculant on brix rather than 10 ppm, although this difference disappears at higher temperature.

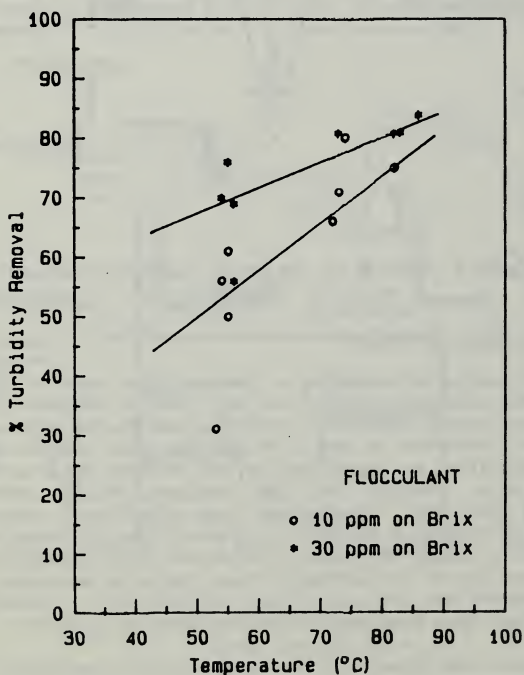


Figure 7.--Effect of temperature and flocculant dosing level on performance of the Empangeni syrup clarifier

In general, it was found that the syrup clarifier was always able to achieve the same degree of turbidity removal as that obtainable in the laboratory, of the order of 85% removal. Subsequently it has been found this provides a useful check as to whether the plant syrup clarifier is performing as well as it should, by comparison with the results obtained in the laboratory.

From being in the position where the mill was regularly unable to produce VHP sugar, Empangeni with syrup clarification was able to produce consistently good quality sugar meeting VHP specifications. In addition, for the first time in many years, the mill was able to achieve molasses exhaustion results comparable with the best mills in the industry.

#### Felixton Syrup Clarifier

A 45 m<sup>3</sup> syrup clarifier has been in operation continuously at Felixton mill since it was started up. Because of an unsatisfactory syrup heating arrangement, the clarifier was generally run at lower temperatures of around 60°C. Under these conditions, turbidity removal was lower, of the order of 60 - 70%. The syrup heating problem has recently been overcome and turbidity removals of over 80% are now possible.

In spite of the fact that turbidity removal was lower than it should have been, no problem was experienced in making VHP specification sugar, even when mixed juice purities were as low as 75. Although it is believed that the continuous A-pans at Felixton also assist in reducing sugar colour, the syrup clarifier has ensured that sugar quality has been consistently acceptable. During a period of two weeks when the syrup clarifier was taken off range, an immediate deterioration in sugar quality was evident where the sugar is received at Hulett's Refinery.

#### Triangle Syrup Clarifier

The major problem facing Triangle white sugar quality was the presence of suspended solids in the sugar. White sugar is made by remelting high pol sugar (at 99 pol) and re-crystallising. In order to remove suspended solids, remelt was screened through a Sweco rotary screen and then through a very fine nylon mesh. This arrangement still did not cure the problem, and also required that the remelt brix be reduced in order to achieve adequate throughput through the screens.

A syrup clarifier was installed, which has had noticeable benefits, and regularly achieves average turbidity removal of about 85%. Syrup clarification has been particularly effective in removing bagacillo from raw sugar. The 120 mesh screen on the Sweco screen has been replaced by a coarser screen which simply removes large particles of extraneous matter. Secondary

screening has been dispensed with and the brix of the remelt has been able to be raised to improve steam economy. Suspended solids in white sugar is now better than it was prior to the installation of the syrup clarifier.

The decision to install a clarifier on raw syrup rather than on melt was made so that the downstream ethanol plant would benefit from the cleaner molasses feedstock.

## DISCUSSION

It is apparent from the results that conditions for flotation clarification need to be optimised for each individual application. The choice of polyelectrolyte flocculant, whether additional chemicals are worthwhile and the brix at which the process operates, all depend on the particular characteristics of the material to be treated. The opportunities and advantages of using the process are dependent on the local circumstances prevailing.

### Applications in the Raw Sugar Mill

Clarification of filtrate from rotary vacuum filters is feasible but the process appears to be more temperamental in that a stable scum is not always obtained. While there must be an advantage in not recycling filtrate back to mixed juice, the benefits are not clear. It is therefore difficult to justify the installation of a filtrate clarifier of this type. The capacity of the clarifier and the need for a surge tank ahead of the clarifier largely diminish the advantage of reduced overall retention time, and an additional chemical cost for phosphoric acid is incurred.

Syrup clarification on the other hand is characterised by the stable scum which is obtained. The process is simple, requires little supervision, and the operating cost is low since it requires only the use of flocculant and no additional chemicals. A further advantage is that the scum can be directed back to mixed juice or perhaps to clarifier muds. When the latter option was tried at Empangeni, this resulted in an increase in the pol of the cake, but this could be minimised by optimising the operation. The major benefit to be obtained from syrup clarification is an improvement in sugar quality and a reduction in massecuite viscosities. The advantages of this application are greater when the quality of cane being processed is poor.

A comprehensive investigation into conventional juice clarification was undertaken at Amatikulu (Scott et al 1988). This showed that however good juice clarification may be, there is a large increase in turbidity of about 80% across the evaporators. This indicates that a syrup clarification process should be more beneficial than going to elaborate steps to improve primary juice clarification.



An additional improvement in this process can be achieved if justifiable through the use of lime and SO<sub>2</sub> sparging. Since the precipitate formed is floated off, no downstream filtration should be required.

Clarification of B-molasses instead of syrup has the advantage that the quantity to be treated is small and so a smaller clarifier can be installed. It also seems to have the potential to remove larger quantities of impurities, and will achieve benefits relating to lower final massecuite viscosities and improved recovery. However, compared to syrup clarification, it does not achieve the same improvement in sugar quality, and requires more steam for re-evaporation of the diluted molasses and a solid bowl centrifuge or other system for desweetening the scum.

Clarification of final molasses is sometimes advocated as a means of improving alcohol yields and yeast quality. Flotation clarification of this type could well achieve this objective.

#### Refinery Applications

Some reduction in turbidity and colour can be achieved if the standard flotation clarification process is applied to Refinery melt. However, it has been established that the addition of a small amount of lime and gassing with either CO<sub>2</sub> or SO<sub>2</sub> prior to flotation clarification can give some encouraging results. While the process has not been optimised in the laboratory, it would appear that a colour removal of around 50% is achievable at a much lower lime usage than conventional carbonatation, i.e. 0.05% compared to over 0.5%, and the necessity for an expensive filter station is eliminated.

This process could possibly be highly cost-effective in a refinery attached to a raw sugar mill, particularly since the scum removed can be recycled to the raw house. Otherwise the scum has to be desweetened through the use of a solid bowl centrifuge or further flotation clarification steps. In this case, the quantity of cake produced because of the lower lime addition level will be significantly reduced compared to conventional carbonatation. However, if a high quality white sugar is to be produced, it is unlikely that the need for polish filtration will be eliminated.

The potential for clarifying sweetwater by this technique would have to be assessed in the particular individual situation.



### Type of Colour Removed

The work reported here has made use of colour and turbidity measurements based on light absorbance. This gives no information on relative quantities of the different colour bodies present. An extension of this work would be to investigate what type of colour is removed during the different flotation clarification processes.

Godshall and Clarke (1988) has shown that the high molecular weight colourant is preferentially retained in processing and ends up in the product sugar. If these processes remove some of this high molecular weight colour, their value would be considerably enhanced.

Experience with full-scale syrup clarifiers has shown that although there is little measurable colour change in syrup during clarification, a significant reduction in the colour of the raw sugar is obtained. Godshall (1988) also reports that the very high molecular weight colourant is associated with polysaccharide. Since a reduction in gums in raw sugar has also been obtained by syrup clarification, it is possible that the process removes, together with turbidity, a greater proportion of the high molecular weight colour bodies.

### CONCLUSION

Syrup clarification is a cheap, versatile and simple process which can be applied in a variety of sugar processing applications, both in a raw sugar mill and in a refinery. Roughly eight years of experience with full-scale syrup clarifiers in raw sugar mills has been accumulated. This shows clearly the beneficial effect of syrup clarification on raw sugar quality and massecuite viscosities.

A laboratory procedure for assessing flotation clarification has been developed by which other applications have been studied. The good correspondence between results in the laboratory and full-scale syrup clarifiers suggests that the laboratory work presented here gives a reasonable estimate of results achievable in practice. The laboratory facility also gives the opportunity to optimise the process on a small scale before going ahead with a new installation.

The results applied to flotation clarification of Refinery melt look particularly encouraging and could be applied with benefit in a full-scale installation.

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## DISCUSSION

Question: Can you tell me how the turbidity was measured?

Rein: Turbidity was measured by the difference in absorbance between a filtered and an unfiltered sample, at 720 nm, rather than at 420 nm. At 420 nm there is a great dependence on pH. At 720, results are more reproducible.

Question: Can you give us some idea of the time scales involved, on aeration and scum removal, for example?

Rein: The laboratory test took about 25 minutes. The sample was treated with lime and gas to achieve the required pH, then aerated for 3 minutes.

Question: What molecular weight range was the polymer?

Rein: About 10 million daltons.

Question: Was there much variation encountered in the dosage level on an hourly or daily basis?

Rein: Generally, the polyelectrolyte dosage rate was left unchanged. We had to increase the polyelectrolyte dosage when there was a heavy loading of solid or particulate matter in the syrup. With poor cane quality, we saw a lot more particulate matter.

Question: You showed some numbers on increased syrup purity. Do you have any numbers on increased recovery or production?

Rein: That's very difficult to determine because of the time scale in a raw sugar mill--it takes a week for syrup to go through to final molasses. That's why we chose two week periods of on/off operation, looking at boiling house recovery in the second week of each period. Only a small number of data points can therefore be obtained in a season, and the effect on boiling house recovery could not be established.

## STRUCTURE OF COLORANTS

Margaret A. Clarke, W.S.C. Tsang and M. A. Godshall

Sugar Processing Research, Inc.

## INTRODUCTION

The classification of colorant compounds in both cane and beet sugar manufacture into plant-derived and process-generated or factory-generated groups is well known. Compounds that originate in the growing plant include flavonoids, substituted phenolics, polyphenolics, amines and amino acids; colorant molecules are found in these groups or in their reaction products. Compounds of plant origin have been further classified by molecular weight range and by their tendency to be occluded preferentially in the sugar crystal (Clarke et al., 1984; Clarke et al., 1987; Kofod-Neilsen et al., 1980; Mantovani et al., 1985; Shore et al., 1984; Smith and Gregory, 1971; Tu et al., 1977).

Recently, work at S.P.R.I. has concentrated on a group of cane sugar colorants of very high molecular weight, of plant origin that are intractable in process--most factory and refinery processes fail to remove a significant amount of this group, which continues through process to end up in the sugar crystal. This type of compound shows both polysaccharide characteristics and visible color (Clarke et al., 1987; Godshall et al., 1987).

Although this type of color is not intense, it is expensive to the processor: It has reduced the efficiency of every process in the factory and refinery without being eliminated, while consuming energy and process chemicals, and so has lowered the overall efficiency of production.

In this paper, the structure of this type of polysaccharide-colorant will be discussed. Similar compounds have been found in plants closely related to sugarcane, and potential polysaccharide-colorant complex molecules have been reported in sugarbeet.

## PLANT CELL WALL POLYSACCHARIDES

The polysaccharide known as I.S.P. (Indigenous Sugarcane Polysaccharide) is an arabinogalactan, with glucuronic acid residues (Roberts et al., 1976). I.S.P. is soluble and travels through cane sugar processing to end up in even the highest quality refined white sugar (Roberts and Godshall, 1978).

There has been considerable research into these cell wall polysaccharides in corn and wheat, both members of the Gramineae family to which sugarcane belongs. In corn and wheat, the pentosans are also important as polysaccharides in end product. In sugarcane, the major pentosan is a polymer of xylan, the main component of bagasse, the fibrous residue of cane. Because traces of xylose are often found with I.S.P., it is not clear whether soluble pentosans exist in sugarcane, or whether there is some xylose in the glucuronoarabinogalactan (I.S.P.) structure. The latter is the more likely case.

The major cell wall polysaccharides in the grasses are this type of compound and glucans. The polysaccharides are cross-linked by a variety of mechanisms to provide stability for the cell-wall structure. These macromolecular associations probably include glycosidic and non-glycosidic covalent linkages, hydrogen bonds, ionic connection and a variety of intramolecular attractive forces; structure elucidation is in the early stages. However, it is known that some small substituted phenolics can serve as cross-linking components between cell wall chains. One of these is ferulic acid, shown in Figure 1. This, in corn, is esterified to the O-2 position of the arabinose residues (Nevins and Kato, 1984), as shown in Figure 2. Ferulic acid can cross-link in the plant by dimerization, catalyzed by peroxidase in the presence of  $H_2O$ , as shown in Figure 3.

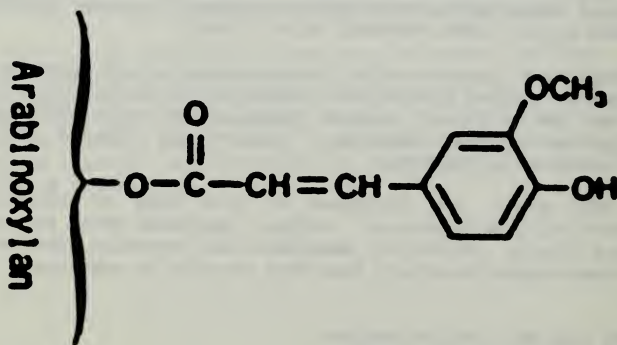


Figure 1.--Ferulic acid esterified onto arabinoxylyan backbone in corn.



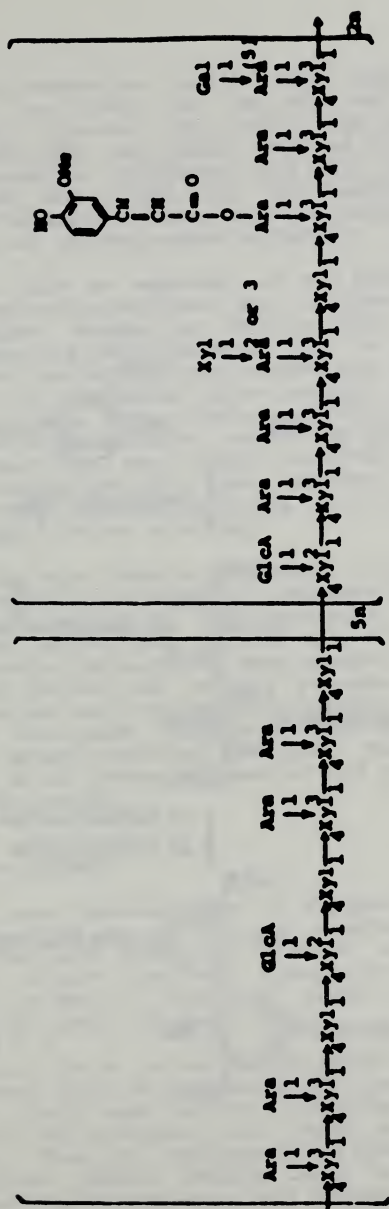


Figure 2.--Corn glucuronoxarabino xylan backbone with ferulic acid ester.

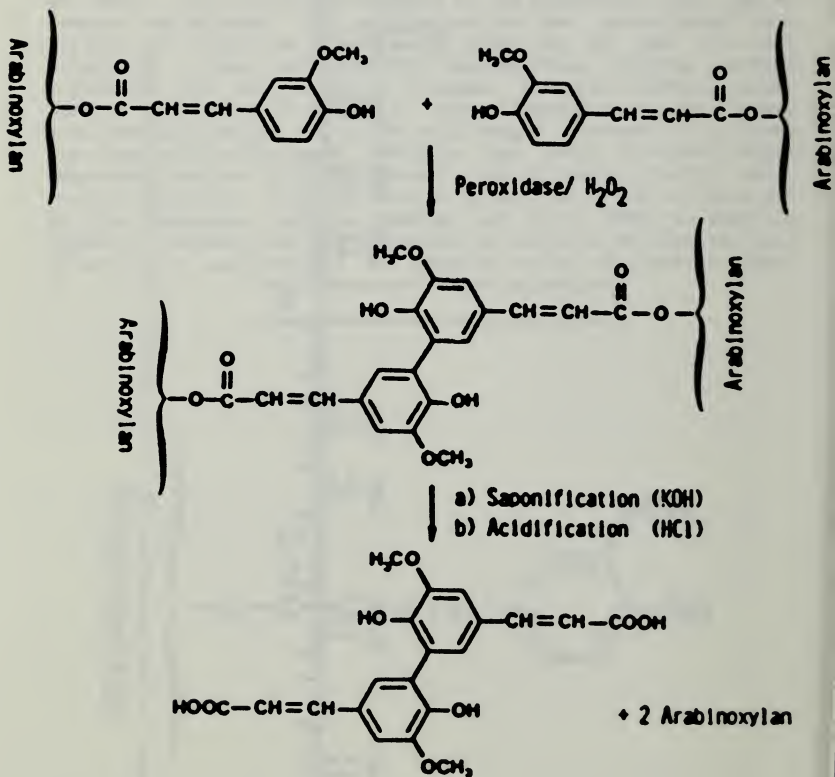


Figure 3.--Dimerization of ferulic acid by peroxidase enzymes to create cross-linking.

These cross-linkages are easily broken when the plant undergoes processing and part of the cell wall polysaccharides are solubilized. However, the ferulic acid ester linkage is less easily hydrolyzed and remains attached to the backbone even when the polysaccharide is degraded into small units. It is proposed (Nevins and Kato, 1985) that the phenolic acid preserves the glycosidic linkages in the immediate vicinity of its point of attachment and prevents enzymic hydrolysis.

To relate this to sugarcane and cane sugar processing: Ferulic acid is a phenolic constituent that is always observed in raw and refined sugars (Godshall and Roberts, 1982). The constituents in these studies are identified after acid or base extraction which could break the ester linkage and release ferulic acid. Ferulic acid is a very pale yellow active compound that can readily react in sugar to form darker colored compounds. In studies on behavior of colorant components model systems for sugar boiling, Devereux (1980) found that when ferulic acid was added to a white sugar liquor, crystals from that liquor developed a yellow color after only a few days, thereby identifying ferulic acid as a compound that causes sugar to darken in storage.

Roberts also noted (Roberts and Godshall, 1978), in a study on the behavior of high molecular weight components in sugar refining, that material that appeared to be polysaccharide, isolated from refined sugar, when treated with ethanol, released some colored compounds. The observation indicates that some colorant can travel through all raw sugar factory and refinery processes while associated with polysaccharide covalently attached, probably since hydrogen bonding or other non-bonded interactions seem unlikely to resist the prevalent pH and temperature conditions. These observations can be explained by the presence of phenolic acid groups esterified to cell wall polysaccharides.

#### BEET SUGAR COLORANT

For many years, polysaccharides in sugarbeet and beet sugar have been classified as pectins, and relatively little work has been done on them until recently. The advent of sugarbeet fiber as an important dietary fiber has spurred further research. J. P. Thibault, at INRA, at Nantes, in France, has made a systematic study of beet pectin (Thibault, 1988).

Beet pectin is a soluble polysaccharide, composed primarily of galacturonic acid and monosaccharide residues. Rombouts and Thibault (1985) have recently identified ferulic acid groups, esterified onto galacturonic acid units of the rhamnogalacturonan backbone. These are analogous to the abovementioned ferulic acid-hemicellulose complex; ferulic acid in sugarcane could be esterified onto the glucuronic acid residues of ISP.

It is proposed, therefore, that some colorant and color precursors in both cane and beet factories travel through process attached to polysaccharide; the complex is soluble; in crystallization it preferentially enters the crystal and could, upon storage, especially in conditions of high temperature, de-esterify to release colorant. The de-esterification might also occur in process: high alkalinity is likely to break the ester linkage. The colorant thus formed is no longer associated with polysaccharide and so less likely to cocrystallize with sucrose. It has often been observed that, in a comparison of fine liquors from carbonatation and phosphatation processes, sugars of equal whiteness can be crystallized from carbonatated liquor of much higher color than phosphatated. Carbonatation conditions use a pH high enough to hydrolyze the ferulic acid groups off the polysaccharide, whereas phosphatation conditions do not. Hydrolyzed phenolic acids would contribute to syrup or liquor color, but less to crystal color. It is therefore possible that this type of colorant provides one contributing factor to the observed differences in crystallization behavior from carbonatation and phosphatation liquors. Alkaline decomposition of invert also contributes to this phenomenon, of course, and a quantitative study is required to determine relative contributions.

Preliminary work at S.P.R.I. on high molecular weight color and polysaccharide behavior in beet juice with varying carbonatation conditions shows that at higher pH (11), more total color is removed than at lower pH (10), but a higher proportion of remaining color is low molecular weight at pH 11 than at pH 10. Some of this color, as abovementioned, comes from invert decomposition, but some may be de-esterified phenolic colorant.

It is also possible that cross-linked hemicellulose, as shown in Figure 3, is a part of the polysaccharide-colorant complex material.

Other workers have recently emphasized the growing importance of high molecular weight colorant in beet sugar. Broughton et al in 1987 reported observations on HPLC separation systems of colorant at 40,000 daltons. It was suggested that this colorant is formed in process from polymerization of smaller molecules.

Vogel and Schiweck (1988), of Suddeutsche Zucker, have recently begun an investigation into high molecular weight material in sugarbeet, and reported from 0.13% - 0.14% by weight of polysaccharide material greater than 10,000 daltons. In their gel permeation studies, a peak at 100,000 daltons was observed in thin and thick juice and molasses, and another peak at 200,000 daltons in raw juice, both with colorant and polysaccharide nature. These were attributed to hemicellulose from the beet.



## CONCLUSION

It appears that high molecular weight material from both sugarcane and sugarbeet is of importance in processing both for its polysaccharide characteristics and its colorant properties. Recent observations indicate that this material, known for some years to be a factor in sugarcane processing, may also affect sugarbeet processing.

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## DISCUSSION

Question: A comment, rather than a question. I agree with Dr. Clarke that there are a number of colorants of interest to both beet and cane. In the 1987 campaign, we carried out in Ferrara, in cooperation with the sugar company Eridania, some investigations concerning color precursors. To our surprise, we found, along with polyphenolics in sugarbeet juices, also flavonoids--apigenin and other compounds. This work was published by Dr. Maurandi (Indus. Sucr. Ital. 1988 (3)).

Clarke: That paper contains some very good work.

Question: You talked about the combination of colorant and polysaccharide at high molecular weight which must be in the millions. How do you believe that such an enormous molecule can be preferentially taken into the sucrose crystal?

Clarke: I can certainly explain how any complex that has a dextran in it can be taken into the crystal. The sucrose molecule, that is the beginning of any dextran molecule, will fit right into the sucrose lattice. Other glucans have at least glucose as one terminal group.

Question: A couple of points: First, on the color of feruloylate esters--it's easy to believe that water-soluble polymers carrying feruloylate esters can be extracted from Gramineae. On the question of their color--this will be extremely sensitive to pH.

When the parahydroxy group ionizes, you get an extended sequence of conjugated double bonds, and therefore color. That's the yellow color that you always get with paraphenols of the cinnamic acid group. So when you get materials showing more color in alkali, you suspect the parahydroxy group.

In answer to another question, the proanthocyanidins are colorless, the same as leucoanthocyanins, but develop red to orange colors in acid.

Clarke: Those compounds have been found in raw sugar. It seems to me we've found these involved in plant protection systems also--perhaps Mrs. Godshall will comment on this.

Godshall, S.P.R.I.: We have found luteolinidin (3-deoxyanthocyanin)--that is orange-colored. We also did some work that was never published, some years ago, on leucoanthocyanins in cane juice. We were able to extract them and, upon acidification, obtain a red color. Perhaps we should follow up on this--the compounds are definitely in cane juice.

Clarke: This all emphasizes that both cane juice and beet juice contain plant extracts in very complex systems.

# NEAR INFRARED SPECTROSCOPY IN SUGAR ANALYSIS. BEET SUGAR MANUFACTURING.

G. Vaccari and G. Mantovani

University of Ferrara, Ferrara, Italy

## INTRODUCTION

In previous experiments (Vaccari et al. 1987), we examined the possibility of using NIR (near infrared) techniques in sugar analysis, and, in particular, for factory chemical control. On the basis of preliminary results, ICUMSA has recommended further study of this technique bearing in mind the rapidity of carrying out the relevant analyses (Mantovani 1986). In fact, as is known, NIR can be performed with a minimum of sample handling and allows the simultaneous determination of different parameters on the same sample.

Burzawa and Melle (1988) recently carried out a series of experiments confirming the validity of the NIR technique in the analysis of various juices from the sugar industry. Sverzut et al. (1987) and Chou (1988) recently applied this technique successfully.

However, the favorable results we obtained needed to be confirmed regarding the possibility that in the sugar factory the same calibration curve set up in previous years may be utilized, with some minor and rapid corrections. The purpose of the present investigation was to confirm this point.

## EXPERIMENTAL AND RESULTS

The same type of instrument already described in the previous investigation (Vaccari et al. 1987), was used. By adopting the calibration curves set up the previous year, and the same type of filters, the polarization of the beet brei and brix and polarization of raw, thick juices and molasses, were determined. The same system of insertion of the samples previously described (Vaccari et al. 1987) was utilized. Bearing in mind the need to compare the whole data, raw and thick juices originating from the sugar factory were indicated by B, and molasses by A as in the previous investigation (Vaccari et al. 1987). In the meantime neither of the sugar factories had changed their technological schemes so that the composition of the relevant juices was not modified in any way that would influence the matrix effect.

Using some preliminary tests we noted that the original calibration curves could not be used as such, but needed an adjustment since the differences between the values of the instrument with respect to those of the traditional analysis were almost constant. Therefore, we corrected the intercept of the calibration curves by using sample sets numbering between ten and twenty. Then, samples taken in different days within the range of the whole campaign were analyzed using the corrected calibration curves. The results obtained were statistically analyzed according to the method used in the previous investigation (Vaccari et al. 1987).

#### Beet Sucrose Content

After the correction of the calibration curve, (5 filters), 37 samples were analyzed. These samples originated from the same area of the beets used the previous year for the setting up of the calibration curves.

In Figure 1, the analytical values obtained with the NIR and the laboratory traditional method are compared. Although dispersions are slightly higher than the ones obtained the previous year the results are more than acceptable from the statistical point of view. This means that the modification of the beet non-sucrose composition from one to another year, and in the same area, is not sufficient to invalidate the calibration curve.

Using the same conditions and corrected calibration curve, another 56 samples were analyzed from beets that originated from very different areas in comparison with the ones used the previous year for the setting up of the calibration curve. Figure 2 shows the results. These, although acceptable from the statistical point of view, show higher dispersions in comparison with the ones of Figure 1. This fact can be better understood if we take into account that beet originating from very different areas presumably have a different non-sucrose composition, the matrix effect of which was not taken into consideration in the setting up of the calibration curve. Therefore, if we have to analyze with sufficient reliability beet originating from very different areas we need to take into consideration also these types of beet during the setting up of the calibration curve.

Raw juice. [Factory indicated by B in the previous experiments (Vaccari et al. 1987)].

Twenty-five samples of raw juices were analyzed using the calibration curve made using two filters for brix and four filters for polarization. The polarization and brix data are shown in Figures 3 and 4, respectively. The results, either concerning the statistical analysis or the dispersion between NIR and laboratory data, compare well with the ones obtained



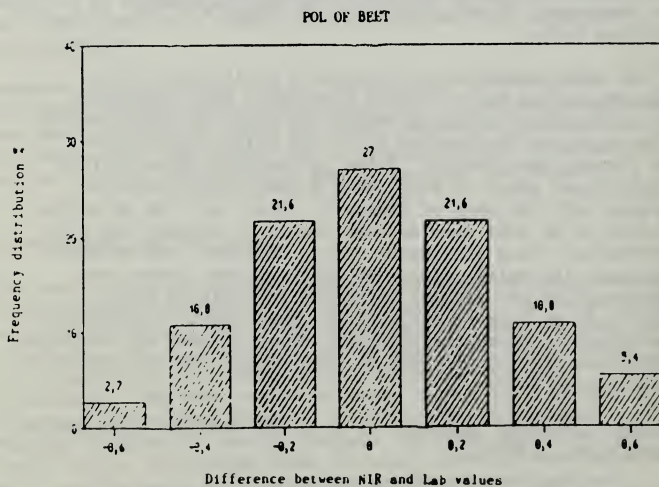
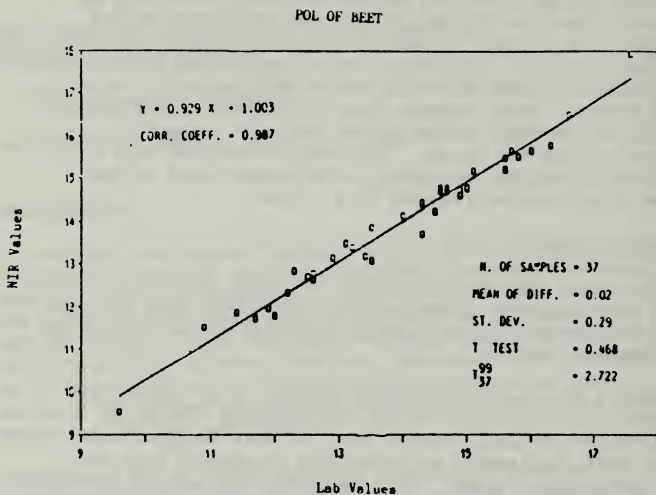


Figure 1.--Sucrose content of beet. Samples originating from the same area of the beets used the previous year for the setting up of the calibration curves.



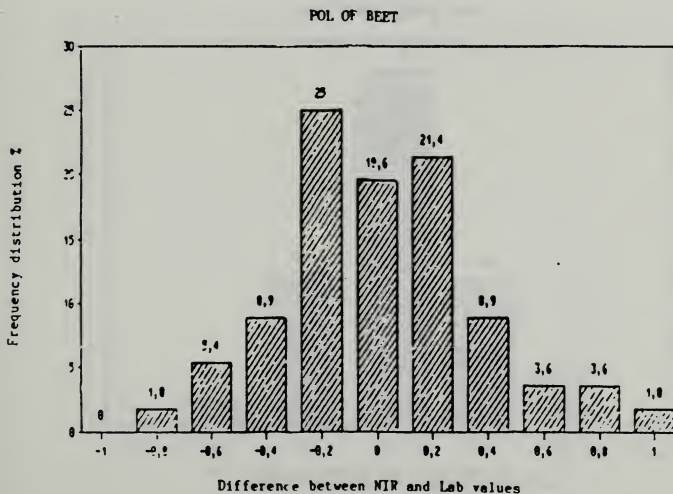
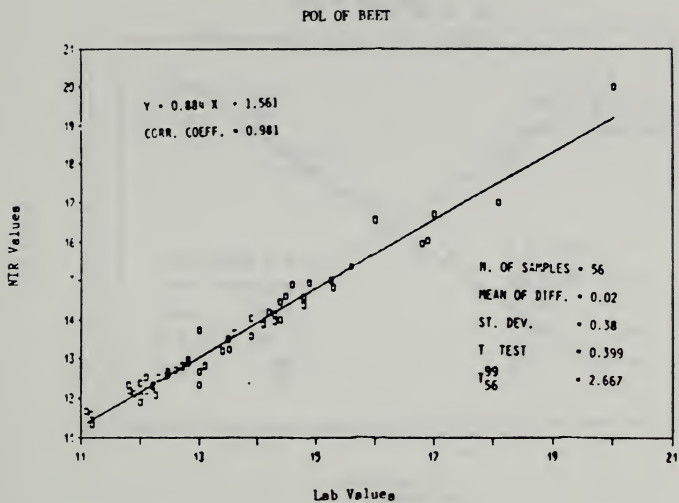


Figure 2.--Sucrose content of beet. Samples originating from very different areas.

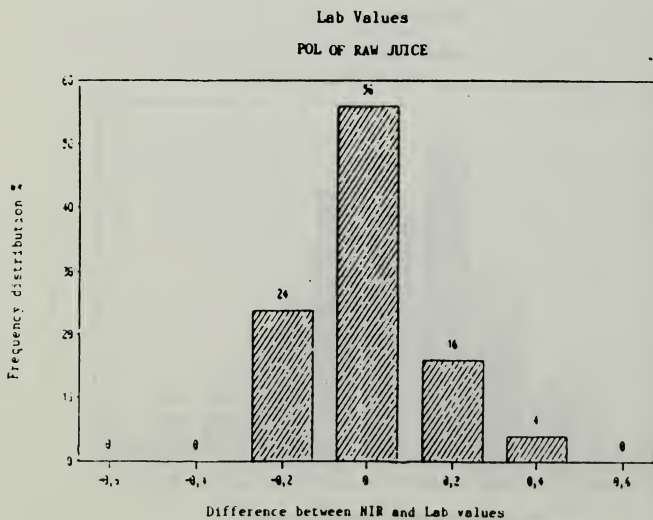
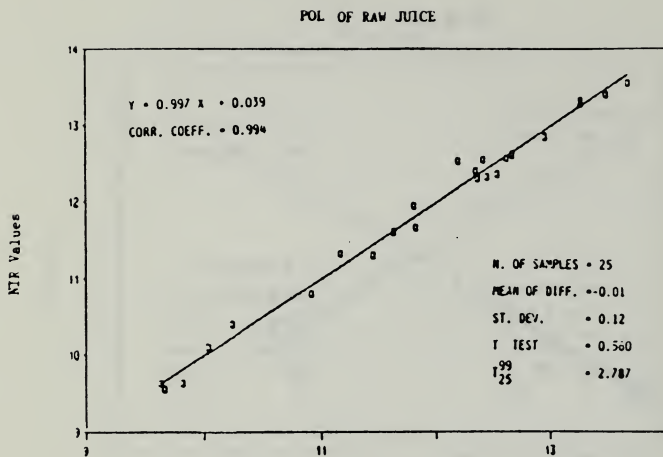
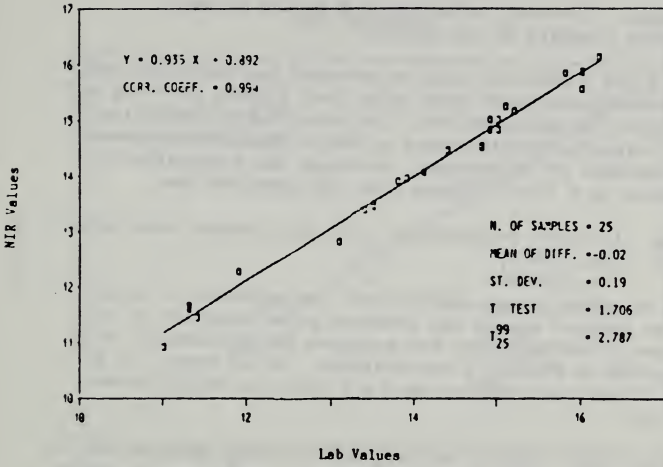


Figure 3.--Sucrose content of raw juice.

# BRIX OF RAW JUICE



# BRIX OF RAW JUICE

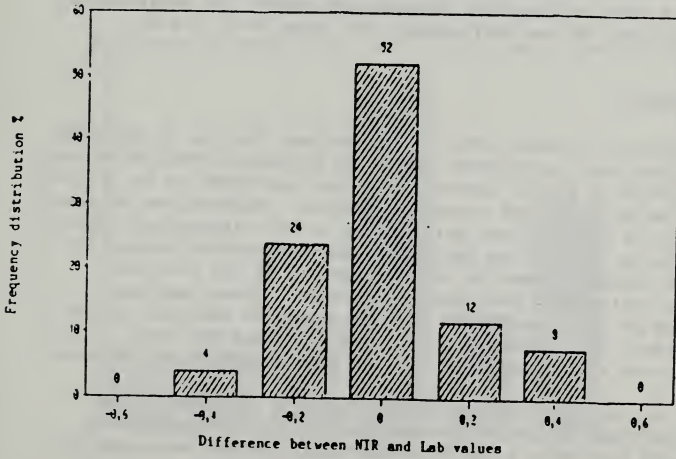


Figure 4.--Dry substance of raw juice.

the previous year. However, a little spreading of the dispersions corresponds to a higher correlation coefficient.

Thick juice. [Factory indicated by B in the previous experiments (Vaccari et al. 1987)].

Figures 5 and 6 show the results obtained for the thick juice with a calibration curve made using four filters for brix and four filters for polarization. The statistical estimation shows no significant difference at 99% of probability between the two methods of measurement, although the dispersion of differences is a little higher than the previous year.

Molasses. [Factory indicated by A in the previous experiments (Vaccari et al. 1987)].

Instead of taking into consideration the molasses of factory B, which, as pointed out in the previous paper (Vaccari et al. 1987), has a Steffen plant for molasses desugarization, only the molasses of Factory A was analyzed. As is known, in a Steffen plant, polarization data are affected by raffinose recycling.

There were three filters for brix and five for polarization for the calibration. The results, (Figures 7 and 8) on the whole do not differ from the ones of the previous year showing slightly lower differences dispersions. Both for polarization and brix, 80% of the differences are lower than  $\pm 0.3$ .

## CONCLUSIONS

On the basis of these results, we conclude that it is possible to use the same calibration curves in different years with the exception of a slight correction of the intercept to be carried out by using a limited number of samples. However, we believe that, for a more correct utilization of the calibration curves, these latter have to be revised in the interseason period by using a certain number of average samples of the previous campaign which have been properly stored. These samples, analyzed by the traditional methods, would be analyzed by the instrument, thus increasing the data available for the calibration which could be, year by year, updated and improved.

As far as the polarization of beet brei is concerned, some problems could arise in the analysis of samples originating from very different areas in comparison with the ones used for the preparation of the calibration curve. In this case the calibration is to be refined taking into consideration samples originating from different areas.

Because of the favorable results obtained we feel that this type of instrument can also be used for on line control of brix and polarization of the various factory products. In one of

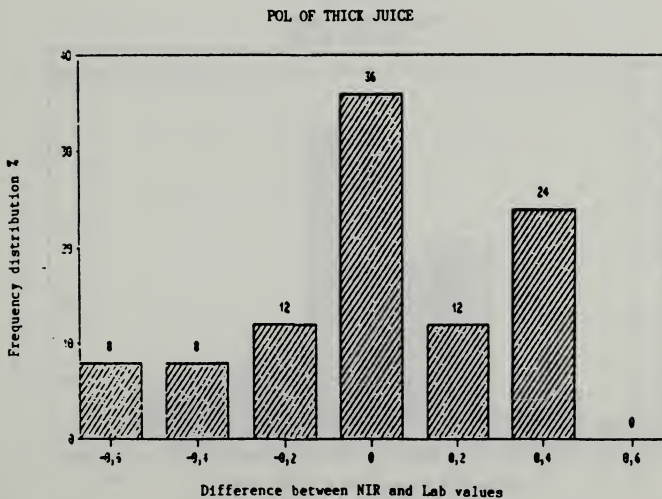
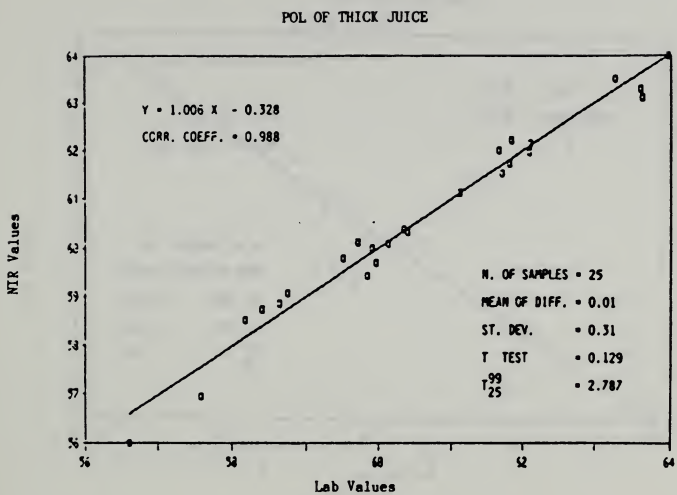


Figure 5.--Sucrose content of thick juice.



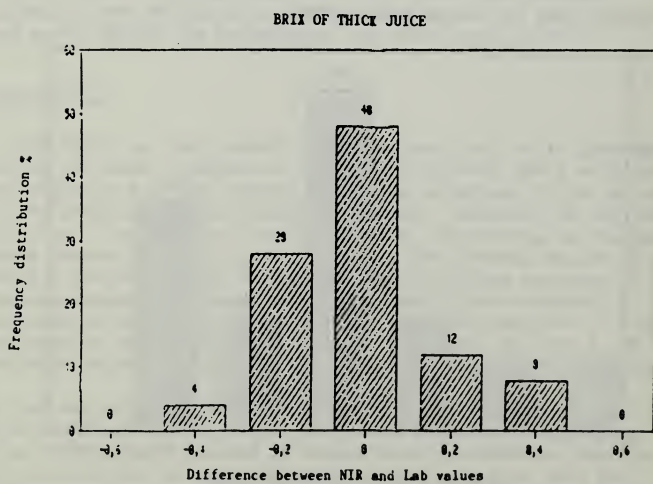
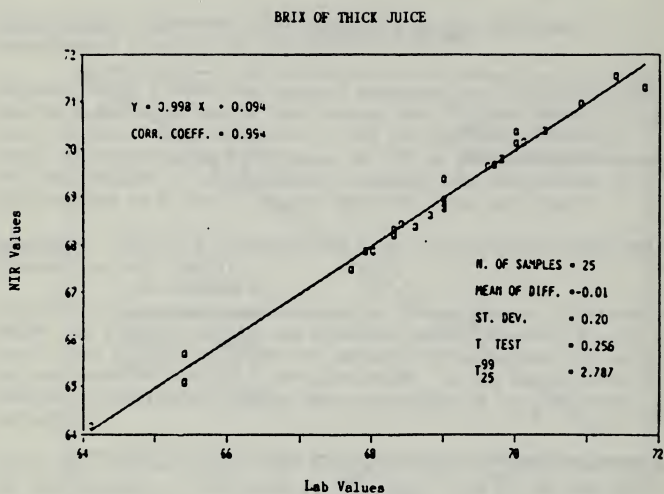


Figure 6.--Dry substance of thick juice.

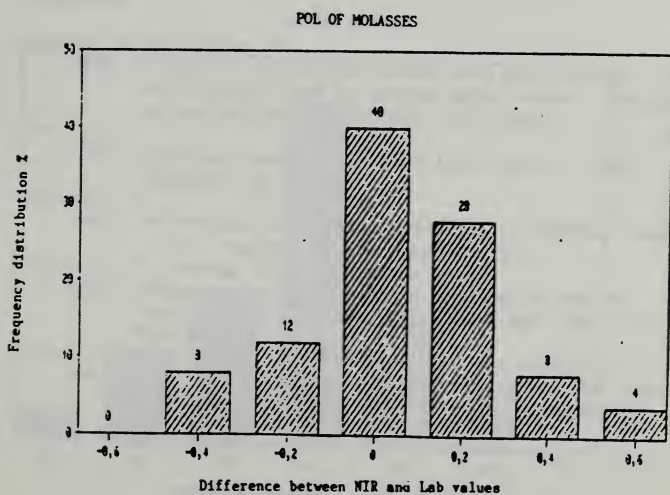
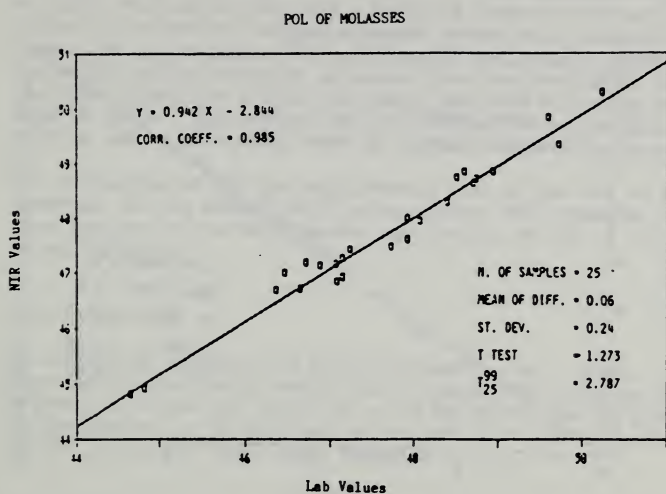


Figure 7.--Sucrose content of molasses.

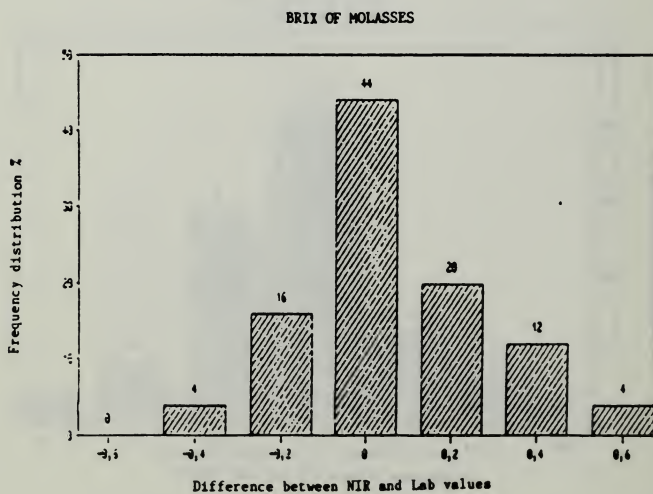
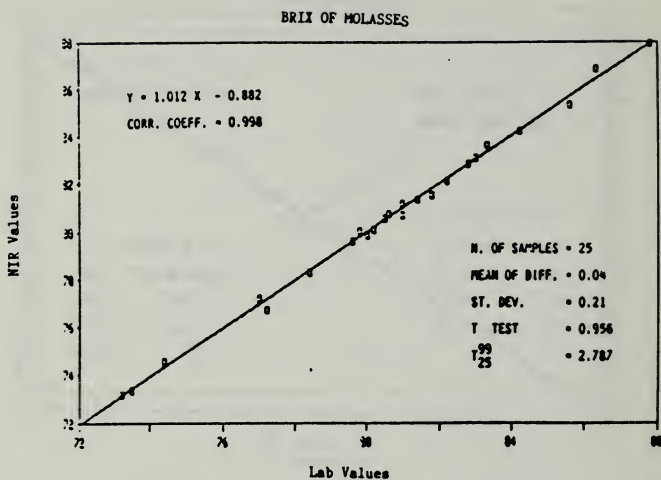


Figure 8.--Dry substance of molasses.

the Italian sugar factories, a NIR instrument is at the moment in operation for experimental purposes. This apparatus employs a single measurement cell and a suitable computer system, and allows us to analyze various types of juices at a programmed rate. The juices enter the cell through peristaltic pumps. An automatic system allows us to insert into the instrument the various calibration curves previously prepared according to the type of juice. The results can be collected by a printing machine and automatically sent to the data collection center.

The evaluation of all the experimental data at the end of the campaign, at the moment still in operation, will allow us to draw conclusions on this further application of NIR in the beet sugar industry.

#### ACKNOWLEDGEMENT

We would like to thank the firm Bran+Luebbe s.r.l. Italy. Moreover we would very warmly like to thank the Direction of Eridania Z.N. and the Direction of CO.PRO.B. for having given us the samples to be analyzed and for the very kind cooperation we had during the whole sugar campaign.

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## DISCUSSION

(The paper and discussion were presented by G. Mantovani.)

Question: When you do these 25 samples for each year, what time period did you use? Did that span the whole campaign? And were the samples frozen?

Mantovani: We gathered samples throughout the campaign. The samples of brei were analyzed immediately whereas the samples of raw juice, thick juice and molasses have been analyzed at the end of the sugar campaign. The samples of thick juice and molasses were stored at room temperature whereas the samples of raw juice were stored at -20°C until they were analyzed.

Question: I have a statistical question. Is it allowed to compare two different analytical methods with only a T-test? ISO has specified methods for comparing analytical results but not the T-test.

Mantovani: Actually, there is only one analytical method, that is the method through which the NIR equipment was calibrated. The purpose of the statistical investigation was to determine how the data given by the NIR technique differed from the laboratory data.

Besides the utilization of the T-test we used also the "CHI-SQUARE-test" and the "PAIRED-DIFFERENCE-test" which confirmed the reliability of the answer given by the NIR technique.

Question: The point is well taken that the question you want to ask in your test is whether the difference in the results is zero. You put a limit on the size of the difference and then asked the question, is it really that big. If you make the T-test tighter and tighter, as the previous questioner suggested, of course it is eventually not going to fit. But what you did do with this test is show that there is a relationship and obviously a very strong one. There are T-tests for goodness-of-fit, which are probably more appropriate ones.

Also, what you did do on your differences was show the distribution of the differences, which was only a couple of tenths. Those results are quite acceptable. So your difference between methods is not as great as, say, three-tenths.

Question: Was there any problem associated with turbidity?

Mantovani: There was no problem with turbidity but we needed to filter the raw juice samples through cotton wool to eliminate suspended and coarse particles.



## NIR ANALYSES OF SUGAR PRODUCTS

Sandra H. Stevens

California and Hawaiian Sugar Company

### INTRODUCTION

Since Karl H. Norris (1962, 1964) first described a near infrared moisture meter for grains, near infrared spectroscopy has become increasingly popular. It is an appealing alternative to cumbersome and time-intensive traditional analytical methods, particularly in the food industry. This paper describes efforts at C and H sugar to develop near infrared, or NIR, methods in order to improve the efficiency of our laboratory operations.

### BACKGROUND

Traditional mid-IR spectra are composed of relatively sharp absorption bands that are characteristic of different functional groups. The positions of these absorptions are affected by the chemical environment of the functional group. Consequently, the mid-IR spectrum of any given molecule is highly characteristic, and has traditionally been used as a tool to elucidate the structure and identification of organic compounds. Although the specificity of the technique makes quantitative measurements in this region theoretically attractive, it has only been since the advent of FTIR, or Fourier Transform Infrared instrumentation, that quantitative measurements in the mid-IR have become practical, primarily because of instrumental design limitations.

The signal-to-noise ratio for non-Fourier Transform mid-IR instruments is low because of low intensity sources and relatively insensitive detectors. Also, as mentioned above, the absorption bands found in the mid-IR region are very sharp, and therefore, in order to accurately measure absorbances in this region, narrow slit widths and high wavelength accuracy are required. Also, since absorptivities are very high in the mid-IR region, very narrow path lengths are required. It is very difficult to construct these narrow path cells with a uniform thickness. The materials required to construct cells that are transparent in the mid-IR region, such as sodium chloride, are difficult to handle since they are easily scratched and damaged by common solvents. It is often difficult to find solvents for

a given application with sufficiently low absorptivities in the spectral region of analytical interest. For this reason, aqueous samples are particularly difficult to handle, as absorptions due to water tend to dominate the mid-IR region. Finally, traditional mid-IR spectral scans are quite time consuming, and typically require five to ten minutes to complete.

The near-infrared region of the electromagnetic spectrum ranges from 750 to 2500 nanometers - falling between the traditional mid-IR region and the visible region (Figure 1). The absorptions found in the near-IR region are the overtones and combinations of the fundamental absorptions found in the mid-IR region. Consequently, near-IR spectra are not composed of the sharp, well-defined peaks characteristic of the mid-IR region, but are composed of broader, relatively weak, overlapping peaks. These weak bands are more influenced by their environment than is the fundamental of the same vibration. Because of these characteristics, the near-IR region is not useful for structural studies, but can be used to obtain quantitative information about a particular substance.

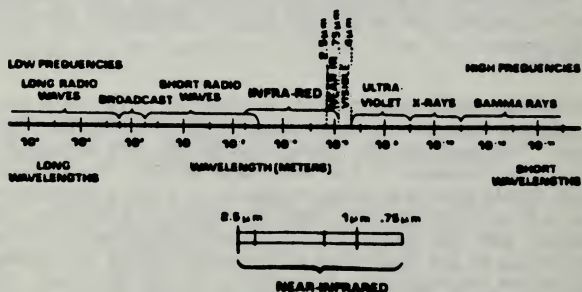


Figure 1. Electromagnetic spectrum

There are advantages in using this area for quantitative analysis. Since the source emits its maximum radiation near the region of interest, this leads to a high energy throughput. This, in conjunction with the fact that sensitive solid state detectors are available, allows the design of near infrared instrumentation with signal to noise ratios of 10,000:1. These solid state detectors have response times measured in microseconds, and allow extremely rapid analyses. Inexpensive and durable optical materials such as glass or quartz are transparent to near infrared radiation, and optical components and cells are, therefore, more easily fabricated than for mid-IR applications. An important advantage arises from the fact that

the absorptivities for near-IR bands are lower by roughly an order of magnitude for each successive overtone. Absorptivities for solvents, particularly water, are consequently lowered enough so as to not pose serious problems in analyses. More convenient sample concentrations and path lengths are possible for near-IR techniques than for those of mid-IR. This means that in many cases, sample preparation is eliminated.

The major disadvantage of the near-IR technique is that it is empirical, and has therefore been viewed as illogical or illegitimate by traditionally trained spectroscopists. There is no mathematical law which distinctly describes the interaction of radiation with a scattering medium containing a heterogeneous distribution of absorbing materials. Instrument readings are therefore, arbitrary, and require calibration with a set of samples designed to "teach" the instrument how to relate near-IR spectral data to sample constituent concentrations. This calibration procedure requires a great deal of analytical time, and is therefore time intensive.

#### INSTRUMENTATION

During the development of his moisture meter, Karl Norris encountered difficulties in making the moisture measurement that were caused by interferences from other constituents, such as protein, oil, and starch. This problem was solved using a computer correlation technique that selected a set of wavelengths for reflectance measurements in the near infrared region that not only eliminated these interferences, but also allowed the analysis of the interfering constituents. These same wavelengths are still used in most commercial instruments today.

The first commercial instrument based on this technique was the Dickey-John GAC launched at the Illinois State Fair in 1971. Soon after the GAC was introduced, Robert Rosenthal developed the GQA based on a different optical system, and founded the Neotec Company to market it. Instrumentation has grown and evolved from these primitive, by today's standards, beginnings to include more manufacturers who offer a variety of instrument designs. These instruments range from sophisticated scanning models to dedicated on-line product analyzers, designed to analyze specific constituents in specific products in a particular control situation.

The instrument used at C and H for this investigation was a Dickey-John Instalab 610 NIR product analyzer. When we began this investigation, we had a strong conviction that NIR techniques could be developed for several applications, but we had no guarantee that they would, indeed, work. We therefore needed an instrument that we could use, on a somewhat long-term basis, to develop and evaluate our procedures. The Dickey-John

Corporation was extremely helpful in this respect, loaning us an instrument, which we later converted to a rental arrangement, and subsequently purchased. We also needed an instrument that was rugged, reliable, and suited for the applications we had in mind. Our investigation of NIR spectroscopy was not intended as a rigorous research project, but as an evaluation of the practicality of this technique in the refinery. The Dickey-john Instalab 610 satisfied these requirements.

The Instalab is configured as shown below (Figure 2). The source is a broadband tungsten - halogen lamp. The beam is collimated and chopped and the wavelength required is selected by the NIR filter. The NIR filters are rotated into place over the sample, and measurements made at each wavelength determined by the filters. An aperture allows only the filtered, collimated NIR radiation to pass through to the sample. Some of the NIR radiation is absorbed by the sample, and the rest is reflected. A detector measures the amount of this diffuse reflected light. The measured reflectance energy for each filter is converted to a logarithm term which is used to develop calibrations to predict constituents in the product of interest.

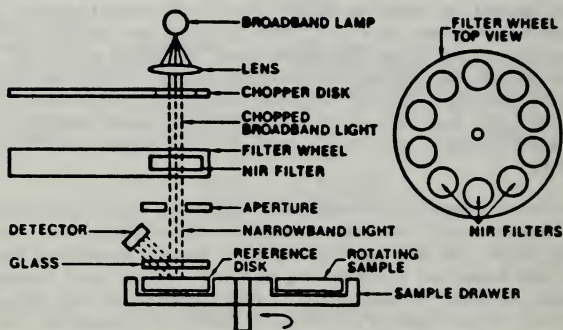


Figure 2. Dickey-john Instalab schematic

The sample drawer has two sections, one for the sample and one for a ceramic reference disc. When the sample drawer is open, between analyses, all the optical filters in the instrument are successively rotated into position above the reference disc. The reflectance data thus generated are the measure of total available radiation. When the sample drawer is shut, during an analysis, the light reflected from the sample at each of the wavelengths is measured again. The logarithm terms used in the



calibration equation are the ratio of sample reflectance data to the reference reflectance data.

Another feature of the Instalab that should be pointed out is its rotating sample cup design. Particle size differences between samples and non-homogeneity within samples are serious concerns in NIR analyses. By rotating the sample holder, the Instalab averages the reflectance readings, and those readings are somewhat less subject to errors caused by non-homogeneity.

The Instalab 610 is also versatile with respect to the filters available for calibrations and analyses. The standard 610 is configured with 10 filters. These filters can be easily changed to provide greater flexibility over a wider range of constituents.

## CALIBRATION

The calibration procedure is the heart of NIR analyses. Calibration is the procedure in which the instrument is trained or taught how to relate selected spectral reflectance data to known chemical analyses. Because this is a mathematical correlation, it is imperative that the data used for calibration are the best available.

Since the NIR calibration is a correlation technique, the error levels associated with NIR analyses can be no better than those of the reference analytical method. Therefore, the first step in the calibration procedure is to determine the standard errors associated with the analytical methods. A convenient measure of the inherent variability of an analytical method is the pooled standard deviation. A set of ten samples is selected and each sample split into half. The duplicate samples are submitted for blind analyses by the reference analytical method. The pooled standard deviation of the method is defined as follows:

$$\text{Pooled standard deviation} = \sqrt{(Id^2/2N)} \quad (1)$$

where  $d$  = differences in duplicate readings  
and  $N$  = number of samples

The next step in the calibration procedure is to select the samples for the calibration set. The samples should be selected so that they encompass the complete range of all constituents present in the material whether these constituents are of immediate analytical interest or not. As mentioned earlier NIR absorptions are influenced by each constituent present in the sample matrix. Unless the NIR instrument has been taught to recognize all possible variations in sample composition, it will not be able to accurately predict the



concentrations of constituents of interest. As a general rule, the range of constituent values for the calibration sample set should be no less than twenty times the pooled standard deviation of the analytical reference method.

Samples should be collected from the same point in the process that the NIR instrument will be monitoring. There can be subtle differences in the composition of products taken from slightly different points in a continuous process. Often times these subtle differences are significant enough to seriously affect the performance of a calibration. If several different points in a continuous process are to be monitored, it is frequently necessary to develop separate calibrations for each control point. Since it is necessary to have as wide a range of constituent concentrations as possible in the material of interest, a good time to collect samples is when the system is upset, or not functioning normally. If samples are collected when the system is in control, then there would be minimum variability of the material of interest.

Every effort should be made to obtain representative samples. All significant variables such as the samples' moisture status, any time or age variations, storage conditions, processing variables, particle size, texture or hardness should be represented.

The number of samples required for a good calibration depends on the material being evaluated and the constituents of that material being measured. Mathematically, it is necessary to include at least as many independent samples in the calibration set as there are spectral constituents in the sample. Often times, though, it is difficult or impossible to know the number of effective spectral constituents in a particular sample. These constituents include not only chemical components, but also physical phenomena such as particle size, and instrumental effects which contribute independently to the measured reflectance data. It is therefore important to include enough samples to address these considerations. There are papers in the literature that deal with this aspect of NIR calibration in much more detail (Honigs, Hieftje, Hirschfeld; 1984, 1985). As a general rule of thumb, however, a minimum of 25 samples is recommended to calibrate for one constituent, ranging up to a recommended minimum number of 55 for a 4 component system.

Sample preparation is another important consideration in the calibration procedure. Since the NIR reflectance data are influenced by sample characteristics such as particle size and shape, moisture status, bulk density and non-homogeneity, it is important to develop a sample preparation protocol and faithfully duplicate that procedure in the preparation of each sample submitted to NIR analysis. Particular care should be taken to document and duplicate any grinding procedure that

might be required in order to obtain satisfactory uniformity of particle sizes in the samples.

The manner in which samples are presented to the NIR instrument for analyses is also a critical part of the calibration procedure. The decision as to which measurement mode is selected depends on the sample characteristics, absorption levels, and instrument capabilities. For our purposes, we worked with powdered or granular materials, and therefore were limited to a diffuse reflectance technique. Sample presentation for diffuse reflectance techniques usually involves packing the powdered material into a sample holder with a quartz window and a spring loaded cap to uniformly compress the sample against the quartz window. This helps insure a consistent level of packing from one sample to the next. Care should be taken to duplicate the packing procedure for each sample.

Often it is a beneficial exercise to perform an analysis of variance for the sample preparation and presentation procedures and thereby identify those points that contribute the most to the overall error of the NIR measurement.

Once the calibration sample set is selected and analyzed by the reference method, the calibration procedure itself is relatively straightforward. The NIR reflectance data at all available wavelengths are obtained for the same calibration sample set. The lab data and the NIR reflectance data are entered into a computer, and a regression program is used to calculate a set of equations for each constituent. All of the equations generated are capable of determining the constituent concentrations from the NIR reflectance data. These equations are then evaluated by examining their ability to predict the composition of each sample in the calibration set.

These predicted values are then compared with the actual values obtained by the reference method. The standard deviation between the actual and predicted constituent values is reported as the standard error of the calibration (SEC). The coefficient of determination,  $R^2$ , is the proportion of total variance in the actual values explained by the fitted line. These are the primary statistical parameters used to select the best calibration equation. It is important to remember that while the coefficient of determination measures the predictive ability of the calibration equation, it only does so relative to the range of lab values in the calibration set. For this reason, it is important to have a wide enough range of values represented in the calibration set in order to give that calibration the required predictive ability. Another aspect of the coefficient of determination is that in many cases it is less important in judging the appropriateness of a derived equation than is the SEC. It is possible for a set of data to have a very strong correlation, but due to the natural range of values expected in

the constituent of interest and the precision of the reference method, result in a prediction error that is less than satisfactory. Common sense and an understanding of the levels of precision in the NIR calibration that are acceptable should guide the choice of calibration equations. In general, a good calibration equation should have a SEC no greater than 1.5 times the pooled standard deviation of the reference analytical method.

In choosing the number of wavelengths to include in the calibration equation, the simpler the equation, the better. There should be a sufficient number of wavelengths to adequately describe the constituent of interest, and these wavelengths should ideally be chosen by virtue of spectral knowledge of that constituent. By adding more wavelengths unnecessarily, the calibration becomes overfitted, and incorporates non-essential information into the calibration equation. There is a position where the error of prediction versus the number of wavelengths used in the calibration is optimized, and that point should be the goal guiding the selection of the number of calibration wavelengths.

An inspection of the calibration constants generated by the regressions can also be revealing. Large coefficients mean higher noise sensitivity. A consideration, then, in choosing the best calibration equation is to choose one in which the sum of the calibration coefficients is minimized. This will minimize the effects of instrumental noise on the performance of the calibration equation. Also, an examination of the t-value associated with each of the coefficients is a measure of the significance of that term in the overall calibration. The regression and data collection package offered by Dickey-john calculates these t-values routinely.

The calibration statistics are the criteria used to judge the calibration equation and to compare the many calibration equations generated in the regression program. As mentioned above, the SEC, or standard error of calibration, and the coefficient of determination,  $R^2$ , are the primary statistical measures of the goodness of fit and performance of the regression equation. F-values or F-Ratios are used to evaluate the robustness or transferability of the calibrations. In the Dickey-john regression program, F-Ratios of greater than 50 are desirable; a robust, transferable calibration has an F-Ratio of 80 or greater; and a calibration with an F-Ratio less than 20 is not recommended for use. Discussions of calibration statistics and procedures are plentiful in the NIR literature, and the reader is referred to these sources for a more detailed and in-depth treatment of this topic (Osborne and Fearn, 1986; Stark, Luchter, Margoshes, 1986).

Once a calibration equation is selected, it is then important to



verify its performance. A prediction set of samples is selected and analyzed using the same criteria as for the calibration set. The recommended number of samples required in the prediction set ranges from 20 samples for a single constituent calibration to 40 samples for a 4 constituent calibration. The selected calibration equation is used to predict the composition of the prediction sample set, and the performance of the calibration is evaluated statistically. In general, the standard error of performance (SEP) will not be as favorable as for the calibration set, and the coefficient of correlation will not be as good. The evaluation as to whether or not the performance of the calibration is satisfactory depends upon the needs of the individual user. Sometimes, particularly if the time savings and material savings are striking, a certain amount of accuracy or perceived accuracy can be sacrificed to gain speed and convenience of analyses.

## EXPERIMENTAL

We chose to investigate the applicability of NIR analyses to two types of products; powdered sugars, and agglomerated sugars. The constituents of interest in these products are moisture, starch, and invert sugars. All of the traditional methods of analysis for these components are time consuming. The gravimetric starch analysis requires approximately one-half hour to prepare the sample and an additional oven-drying time of four hours. The gravimetric moisture determination requires minimal sample preparation time, but results are not available until the sample has been dried for as long as overnight in a vacuum oven. The Lane and Eynon invert determination requires a total sample preparation and titration time of approximately one-half hour, and this analytical method is highly sensitive to individual analyst's performance and requires the preparation and standardization of reagents on a routine basis.

The object of our investigation, then, was to determine whether calibrations could be developed for starch and moisture in powdered sugar and for invert sugars and moisture in an agglomerated sugar that would give adequate accuracy while significantly improving the timeliness of our analytical results.

### Powdered Sugar

As part of our initial evaluation of the feasibility of using NIR methods, a calibration exercise using pulverized sugars was performed. Since, as outlined above, it is important to have as wide a range of sample constituents as possible to obtain a good calibration, we designed a sample set to conform to that requirement. Starch-free pulverized sugar of the same average particle size as the confectioners' sugar of interest was used as a sample matrix. Known amounts of the corn starch used in

confectioners' sugar manufacture were then added and thoroughly mixed to give a set of 10 samples with a range of starch content from 0 to 5 percent. The moisture content of the samples varied in accordance with the starch content, and ranged from 0.09 to 0.33 percent. This gave us a sample set with a wider range of both starch and moisture contents than would ordinarily be encountered in "real world" samples, and would optimize calibration conditions.

Gravimetric starch analyses and oven moistures were performed in duplicate on the ten samples. The NIR reflectance data were also collected for these samples in duplicate. These data were then submitted to the regression routine. One calibration equation for percent starch and another calibration equation for percent moisture were selected. These equations had the following statistical characteristics:

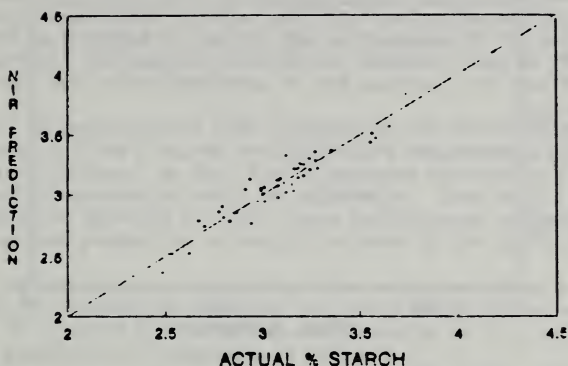
Table 1.-- Initial percent starch and moisture calibration results - simulated confectioners' sugar.

Constituent	R <sup>2</sup>	SEC
% Starch	.990	.142
% Moisture	.991	.010

These initial results were very encouraging, and we therefore undertook a more extensive calibration effort using "real world" samples. Samples of a powdered sugar were collected over several days. The samples were segregated into a calibration and prediction set, and the gravimetric starch and oven moisture analyses performed on both sets. Care was taken to perform the NIR reflectance measurements as soon as possible after the samples were weighed for the moisture determination to minimize the effects of any slight moisture changes in the samples in the interval between the two different moisture analyses. The particle size of the powdered sugar was small enough so that no further size reduction or grinding was necessary. The only sample preparation required was, therefore, packing it into the sample cup.

The regression analysis was performed, and a calibration equation was selected giving the following results for the % starch:





$$R^2 = .930 ; SEC = 0.086$$

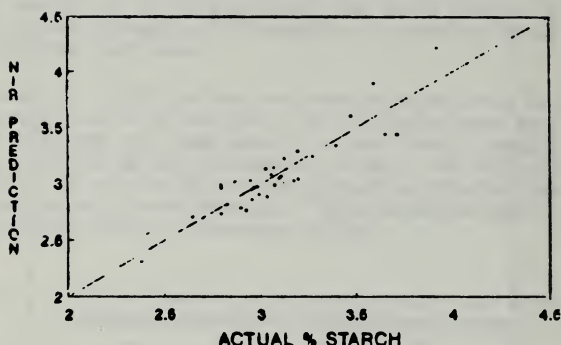
Figure 3.--Actual vs. predicted % starch - Calibration results for confectioners' sugar.

This calibration equation was then used to calculate % starch values for the prediction set. The results shown in Figure 4 were obtained.

As illustrated by these two graphs, the  $R^2$  value for the calibration results of .930 is somewhat greater than the  $R^2$  of .858 for the prediction results. Similarly, the standard error of calibration (SEC) is 0.086 while the standard error of prediction (SEP) is 0.131.

Next, a regression was performed to obtain a calibration equation for the % moisture in powdered sugar. It was surprising to find that the best  $R^2$  values obtained were only in the neighborhood of .55, and that the SEC's obtained were high at .04. After rechecking lab analyses and re-running reflectance data, no improvement in the calibration was realized.

These results were significantly different than would have initially been anticipated on the basis of our preliminary evaluation. While the absolute reasons for this behavior are not known with certainty, some speculation is in order. First, an assumption was made that the simulated samples used in the



$$R^2 = .858 ; SEP = .131$$

Figure 4.--Actual vs. predicted % starch - prediction results for confectioners' sugar.

initial evaluation were an accurate representation of the sample matrix and composition of "real world" confectioners' sugars. This was most likely a false assumption. While we created samples that were a mixture of pulverized sugar and corn starch, other aspects of the sample structure are evidently of spectral significance. One of these differences may be due to the fact that in the "real" samples, the corn starch is added to granulated grinding stock, and the mixture is then pulverized. In our simulated samples, the corn starch was added to the pulverized sugar. It is possible that this difference in composition was significant enough to affect the calibration experiment.

Also, as mentioned previously, the range of sample values used in the calibration effort is just as important as the number of samples. This consideration most likely affected both the % starch and the % moisture calibration, but was of more consequence with regard to the % moisture calibration. While we had comparable % moisture ranges in both the initial and "real world" calibration attempts, there was a major difference in that the initial evaluation contained samples equally spaced throughout the sample range. In our "real world" effort, although the range was comparable, the samples were grouped near the middle of that range with only a very few at the upper and

lower limits. This is an excellent illustration of the importance of designing the sample set so as to optimize the calibration effort.

There are some alternatives that could be used to improve this situation. First, the calibration derived could be used to predict % moisture results, and the results thereby obtained could be averaged to improve the performance of the calibration equation. In choosing this option, however, some of the time savings seen as an advantage of NIR analyses are sacrificed.

After an evaluation of what information was really essential as a result of the % starch and % moisture measurements made for control purposes, we felt that a combined calibration for the % starch plus moisture would be acceptable. Spectrally, this was a sensible approach to take, since the moisture content of a pulverized product is intimately related to its starch content.

We then attempted a calibration that would correlate the spectral information obtained for the calibration set with the sum of % starch plus moisture. The results of this calibration are graphically illustrated in Figure 5.

The calibration equation was then used to calculate the % starch plus moisture content for the prediction set of samples, and the results indicate that this approach is, indeed, workable. Figure 6 illustrates these results.

A comparison of the statistical performance of the NIR methods with that of the traditional methods of analysis is shown in Table 2.

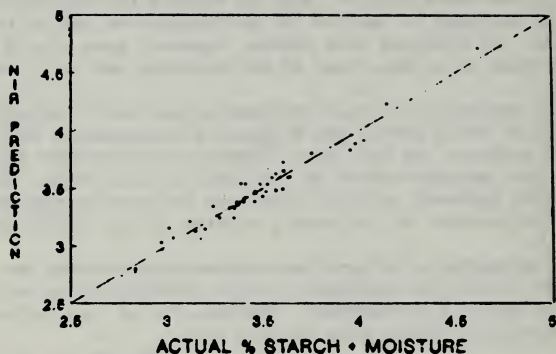
Table 2. Comparison of NIR with traditional analytical methods

Constituent	NIR				Pooled Standard Deviation
	Calibration		Prediction		
	R <sup>2</sup>	SEC	R <sup>2</sup>	SEP	
% Starch	.930	.086	.858	.131	.079
% Moisture	.559	.037	(1)	(1)	.010
% Starch plus Moisture	.959	.074	.919	.105	.080

(1) Prediction not performed

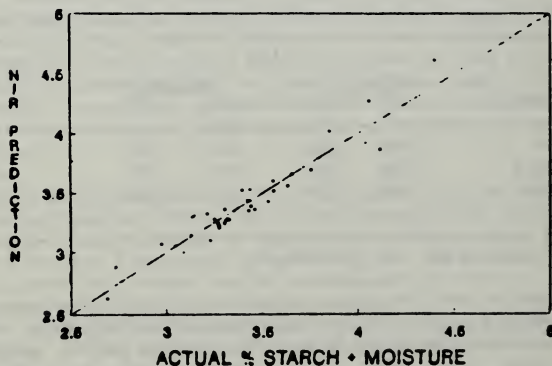
These results show that the combined % starch plus moisture calibration gives a superior calibration statistically, and should be more than satisfactory for routine control purposes.

Next, to evaluate the overall performance of the NIR method for the determination of % starch plus moisture, ten sub-samples of



$R^2 = .959$  ; SEC = 0.073

Figure 5.--Actual vs. predicted % starch plus moisture - Calibration results for confectioners' sugar.



$R^2 = .919$  ; SEP = .105

Figure 6.--Actual vs. predicted % starch plus moisture - Prediction results for confectioners' sugar.

a single confectioners' sugar sample were analyzed using the NIR calibration and the traditional gravimetric starch and oven moisture methods. Table 3 details those results.

Table 3.--Comparison of performance. Traditional methods vs. NIR methods in determining % starch plus moisture in a confectioners' sugar.

Parameter	Traditional Methods	NIR Methods
Average % starch plus moisture	3.84	4.03
Standard deviation	0.03	0.06
Percent error		4.9

By analyzing sub-samples of a single sample, an estimate of the total variance caused by instrumental factors, and by sample preparation and presentation is obtained.

These results show that the NIR method is satisfactory for use as a control method in determining the % starch plus moisture in confectioners' sugars.

Another area of confectioners' sugar analysis that was briefly investigated was that of particle size. Since the particle size of a material affects the magnitude of the NIR absorption at all wavelengths, it should be feasible to develop a calibration that would relate the average particle size to NIR reflectance data. There have been some references to this type of technique in the literature (Czurczak, Torlini, Demkowicz; 1986), (Ilare, Martens, Issakson; 1988). Although we were unable to develop an equation that resulted in a favorable SEP, we did obtain excellent correlation factors. It is feasible that with different software and computing capability, a correlation could be developed which would provide an extremely rapid method for determining the average particle size of materials such as confectioners' sugars.

#### Agglomerated Sugars

We next investigated the feasibility of using NIR methods to monitor the % moisture and % invert in agglomerated sugars. Our agglomerated sugars are prepared by spraying invert syrup onto finely pulverized sugar, mixing, and segregating the appropriate particle sizes of the resulting material. The critical attributes of this product are the % moisture and the % invert.



As was the case for the confectioners' sugar, samples of the agglomerated sugar were collected over a period of a few days. These samples were segregated into a calibration set and a prediction set. Lane and Eynon invert analyses and oven moisture analyses were performed on both the calibration and the prediction sets. NIR spectral data were obtained and fed into the computer for regression analysis. Figure 7 illustrates the calibration equation obtained for the % moisture in agglomerated sugar and Figure 8 illustrates the calibration equation chosen to predict the % invert sugar in agglomerated sugar.

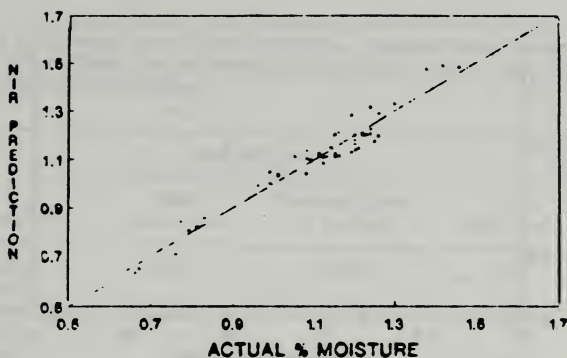
These calibration equations were used to calculate the % invert and the % moisture in the prediction set. Figure 9 illustrates the results for the predicted % moisture and Figure 10 illustrates those for the predicted % invert.

Table 4 compares the statistical performance of the NIR calibration and prediction with that of the traditional methods of analysis.

Table 4.--Comparison of statistical performance. NIR methods vs. traditional Lane and Eynon invert and oven moisture analyses.

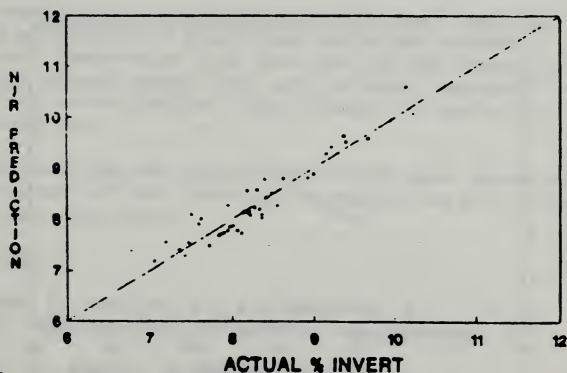
Constituent	NIR				Pooled Standard Deviation
	<u>Calibration</u>		<u>Prediction</u>		
	R <sup>2</sup>	SEC	R <sup>2</sup>	SEP	
% Moisture	.941	.044	.921	.044	.012
% Invert	.903	.233	.884	.270	.116

This table shows that the SEP values are somewhat higher than desired when compared to the performance of the reference methods. However, these reference methods are not convenient methods for control purposes as they are too cumbersome, or time consuming. Our control method for the measurement of % moisture in agglomerated sugars is a Karl Fischer titration. Our control method for the % invert in these products is an automated spectrophotometric method based on the color reduction of a ferricyanide solution by invert sugars. The pooled standard deviation for the Karl Fischer moisture titration in our control operation is 0.09. The pooled standard deviation for the automated ferricyanide invert determination is 0.79. This indicates that the NIR methods are indeed viable alternatives to our control methods.



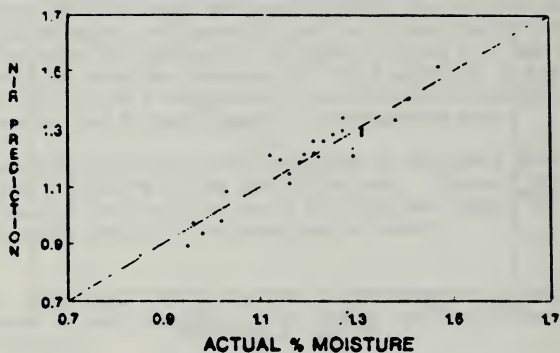
$R^2 = .941$  ;  $SEC = 0.044$

Figure 7.--Actual vs. predicted % moisture - Calibration results for an agglomerated sugar.



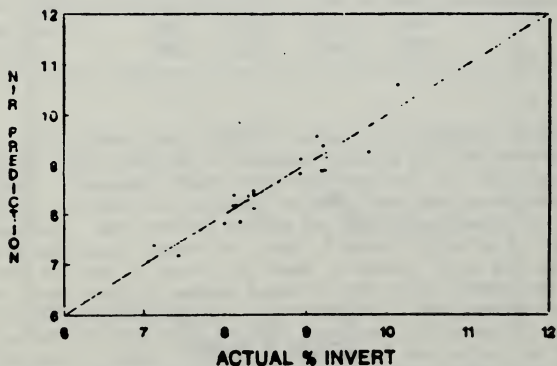
$R^2 = .903$  ;  $SEP = 0.237$

Figure 8.--Actual vs. predicted % invert - Calibration results for an agglomerated sugar.



$R^2 = .921$  ; SEP = 0.044

Figure 9.--Actual vs. predicted % moisture - Prediction results for an agglomerated sugar.



$R^2 = .884$  ; SEP = 0.270

Figure 10.--Actual vs. predicted % invert - Prediction results for an agglomerated sugar.

An evaluation of the overall variance of the NIR method was also performed. In this instance 20 sub-sets of a single agglomerated sugar sample were analyzed for % moisture and % invert content. Table 5 depicts these results.

Table 5.--Comparison of performance. Conventional methods vs. NIR methods in determining % moisture and % invert in an agglomerated sugar.

Parameter	Conventional		NIR	
	Oven Moisture	Lane & Eynon % Invert	% Moisture	% Invert
Average	1.01	8.84	.99	8.48
Standard deviation	.02	.13	.02	.19
Percent error			1.98	4.07

When viewed with an eye toward the time savings and immediacy of response gained by NIR analytical methods for agglomerated sugars, it is apparent that these methods are feasible and indeed attractive alternatives to the traditional methods of analysis for these products.

#### SUMMARY

Our investigation of NIR techniques has shown them to be a viable alternative to traditional analytical methods of analysis. Benefits of this technique include dramatic time savings, reduced reagent use, and increased reproducibility of results by minimizing the "human element" in analytical determinations.

Future investigations should lead to even more innovative and creative uses of this technique to improve the efficiency of laboratory operations.

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## DISCUSSION

Question: First of all, I would like to congratulate you on a fine presentation. What future applications of NIR techniques seem feasible to you?

Stevens: One that is particularly intriguing and that I am investigating is particle size analysis. Some recent work has been done in Norway where the technique is being used to measure the mean particle size (1). When you look at the current methods for screening powdered sugar, this could give a real advantage in terms of time savings. Measurement of invert and moisture in soft sugar may be another application. I haven't tried that yet.

Question: What is the cost of the unit?

Stevens: The unit that we used came in at right around \$12,000, which is very reasonable.

Question: Congratulations on a very understandable presentation. If you will permit a question from a non-analyst and a non-spectroscopist. I note that you use this technique only on very fine materials and powders. If you were to use it on what I would call an ordinary crystal size sample, would the method only see the surface of the crystals?

Stevens: No, there is actually some penetration of the radiation into the sample. As to what sample preparation is required, you have to experiment with it. An interesting sidelight, one of the standard applications of this technique is to measure the hardness of wheat in the grain industry. To do that, they have very rigorously described the grinding procedure. The actual particle size differences that arise when the grinding procedure is followed correlates with the hardness of the wheat.

Question: I suspect that there the particle size effect is due more to the reflectance, as you see more and more of the inside of the grain as it is ground up.

You only referred to solid samples. Is there a difference in principle between using solid samples and liquid samples, like the juices that Dr. Mantovani referred to? In solids you would use reflectance spectrometry and in liquids you would use absorbance spectrometry. Does this make a difference?

Stevens: There are different techniques that you can use depending on the material being analyzed. With the powders, we were using a diffuse reflectance technique. There are also transmission techniques. There is a transreflectance technique in which the beam is transmitted through a liquid and then reflected from a ceramic backing.

Question: I think it is important to realize the limitation of NIR. It is great for in-house control, but for things like comparison of the performance of different factories, payment purposes, or looking at inversion, we need higher precision and higher accuracy. For pol, one needs a precision of 0.05, and NIR is much lower than that. There are restrictions to its use, certainly for payment purposes.

Stevens: I agree. Also the degree to which you want to fine-tune the technique depends, in part, on how much you are willing to invest in it. There are scanning instruments that use first and second derivative techniques that can certainly improve the performance.

- (1) Ilare, J., Martens, H. and Issakson, T.  
Determination of Particle Size in Powders by Scatter  
Correction in Diffuse Near-Infrared Reflectance.  
Appl. Spec. 41(5): 722-8.

## SUCROSE DETERMINATION WITH FLOW INJECTION ANALYSIS IN THE BEET LABORATORY

A.-S. Karlzén and J. Tjebbes

Sockerbolaget, PK, Arlöv, Sweden

In Sweden we occasionally experience problems with frost damaged beets. These beets give problems during processing. It also means that we pay the growers for sugar that does not exist because the sugar analysis for payment is performed by polarimetry, and dextrans from frozen beet interfere with the measurements. We are interested in having a method that can be used in parallel with polarimetry during periods of frost.

There is also another need for a more reliable method of sucrose determination. It is of great interest to have a reliable determination of sucrose content in test beet from seed breeding and agricultural development. We want to be sure that in the future we get beet with high sucrose content; not only high response in the pol measurements due to other components than sucrose.

In Sweden we have a central beet laboratory for all our factories. Since the late seventies we have done a lot of work in automating the laboratory. The brei is clarified with calcium hydroxide and aluminum sulfate, and the digest is analyzed for sucrose,  $\alpha$ -amino-nitrogen, sodium and potassium. Analyses are made both for payment to the growers and for agricultural development and seed breeding purposes. About 200,000 samples are analyzed during every campaign. This is done in four parallel lines in two shifts, about one sample every 30 seconds.

When we started this project we found out early on that an enzymatic method would be preferable to other methods of sucrose analysis (Bergmeyer, 1974; Guilbault, 1976). Three years ago we contacted a research group at the Chemistry Department at Lunds University. This group had done a lot of work with a method for sucrose determination in the presence of glucose using flow injection analysis (Olsson, 1985). Since then we have been cooperating with this group to adapt their research results into a practical method meeting our needs.

The aim for our work was that the enzymatic method should be a part of the already existing system. Other demands were:

- \* The same repeatability as the polarimetric determination.
- \* The analysis should be as fast as one sample/minute.
- \* We wanted the same level of maintenance as for the existing system and of course the method should be reliable in the rather industrial surroundings.

What is flow injection analysis? Flow injection analysis, often called FIA, is a rather new technique for wet analysis. Toward the end of the seventies the first papers on this technique were published in Denmark (Ruzicka and Hansen, 1976, 1981). FIA is based on injection of a liquid sample into a moving nonsegmented carrier stream of reagent. The injected sample forms a zone that moves to a detector dispersing slightly on its way.

A simple FIA system (Figure 1) has a pump that propels the carrier stream and an injection port by which a well-defined volume of sample is injected into the carrier stream. The sample zone diffuses and reacts with the components of the carrier stream in a coil, forming a colored compound which will be sensed by a flow-through detector. A typical recording has the form of a sharp peak, the height of which is related to the concentration of analyte.

Our system is designed in a similar way, but the enzymes used in the analysis are not dissolved in the carrier solution because of the great cost. Instead the enzymes are chemically bound (this is what normally is called immobilized enzyme) (Carr and Bowers, 1981; Guilbault, 1984) on small glass beads. The beads are put into a 200  $\mu$ L reactor. Two different reactors are used in series. The main reactor converts sucrose to hydrogen peroxide by using the enzymes invertase, mutarotase and glucose oxidase.

Hydrogen peroxide is detected by using peroxidase and a chromogenic reagent.

To avoid interference from glucose present in the sample, a pre-reactor is put before the main reactor containing the enzymes mutarotase, glucose oxidase and catalase, which destroy the glucose already in the sample.

Figure 2 is a schematic of the system used in the beet laboratory. Citric acid is used in the carrier stream. The digest is diluted 15 times, and 2  $\mu$ L of digest is injected. The sample passes a dilution coil and then goes through the first reactor. Then the sucrose in the sample is converted to  $H_2O_2$ .



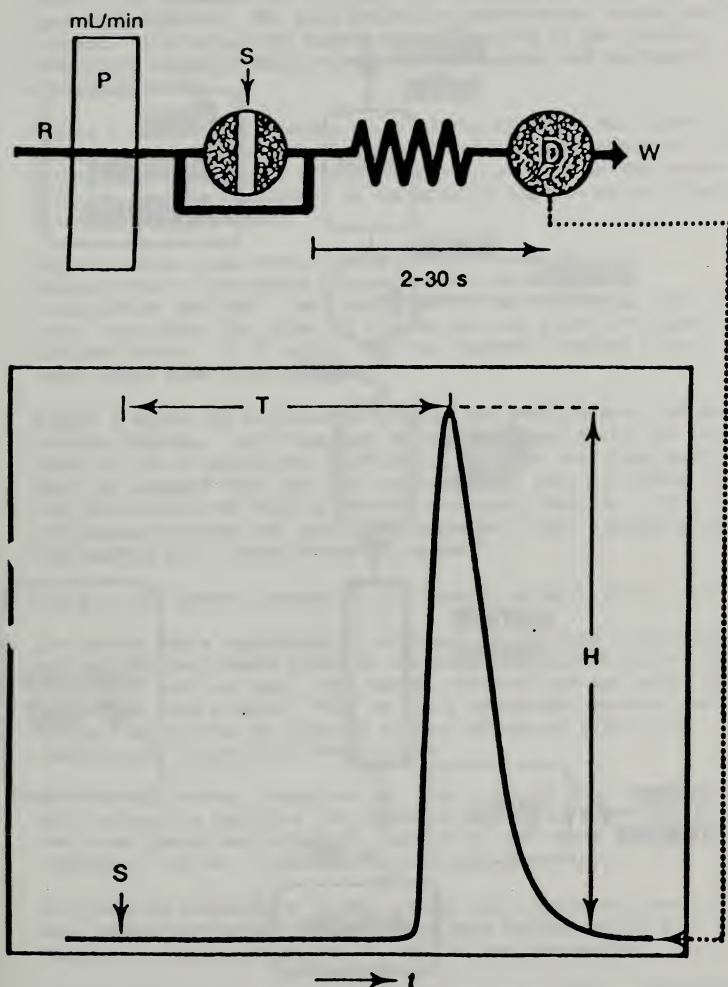


Figure 1.--Top: a simplified diagram of a flow injection system. Bottom: a typical recording of an analysis result.



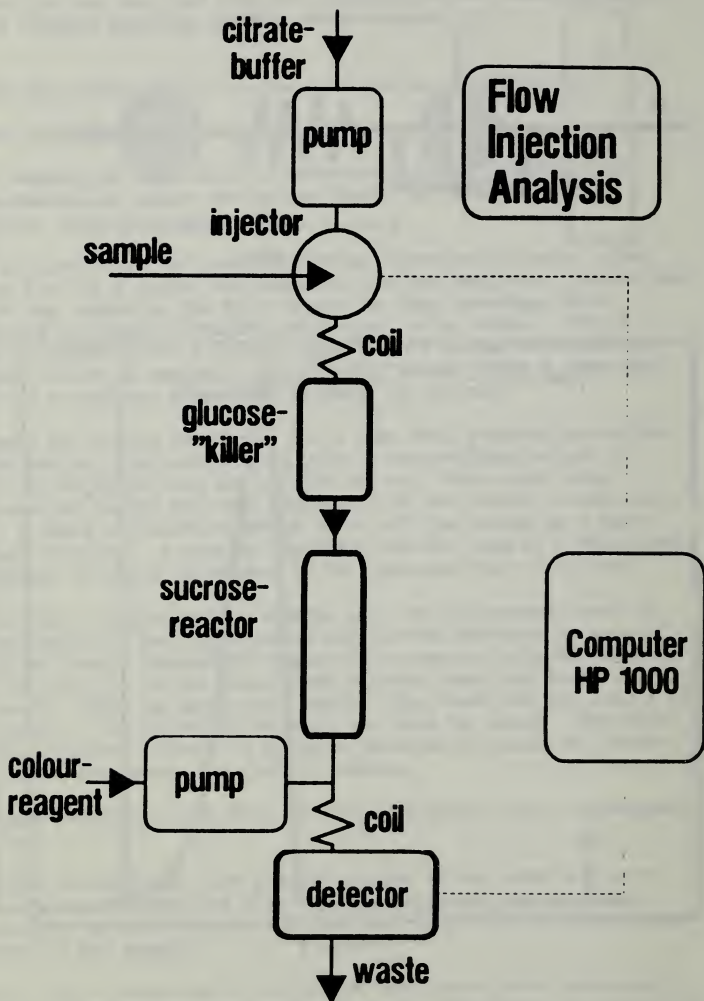


Figure 2.--Schematic of the FIA system used in the beet laboratory.

A color reagent is added at a mixing point. The sample passes through a reaction coil in which  $H_2O_2$  and reagent form a blue color. The intensity of the color is measured in a flow-through spectrophotometer. The peak height is proportional to the sucrose content. The valves and timing are controlled by our regular laboratory computer which also reads the values and calculates the sucrose content.

Figure 3 shows a calibration curve of the system. The curve is linear up to at least 8 mmolar sucrose which corresponds to a Pol-value of 35. At higher levels the oxygen content in the buffer solution becomes limiting. In our diluted digests we are always in the range of 2-5 mmolar.

Figure 4 shows some results from the 1987 campaign. It demonstrates a comparison between sucrose determined by polarization and FIA. The upper line is the regression line. The lower line shows the value if FIA and pol had given the same absolute value. It is clear that the enzymatic method gives a lower value than polarization.

Figure 5 shows the differences between the methods when the digests contain dextran. Last campaign we had no frozen beets, so these are results from a laboratory experiment. We used brei from beet in which we assumed that the cell membranes had been disrupted, and the brei was inoculated with a dextran producing bacteria. The differences between pol and FIA measurements when dextran production has started are clearly shown in Figure 5.

How does the method respond to the demands we have put on it?

The method had a repeatability of about 1% standard deviation. We can analyze one sample every 45 seconds. The system has to be calibrated once an hour. The enzyme reactors and the coils have to be changed once a week. This is fully acceptable because the enzyme reactor can be prepared in the laboratory before the campaign and stored in a refrigerator.

This is the current status of our work. In the next campaign we will attempt to optimize the system further. We also plan to test a few other pumps and injectors, just to be sure that we have the best components for our conditions in the beet laboratory.

For the 1988 campaign we intend to put this system on-line in the beet laboratory to get a larger data base before making a definitive conclusion on how to utilize the new method.

Excluding the computer, the analysis system in this configuration will cost roughly SEK 100,000 (\$15,500).

Calibration curve enzymatic FIA system

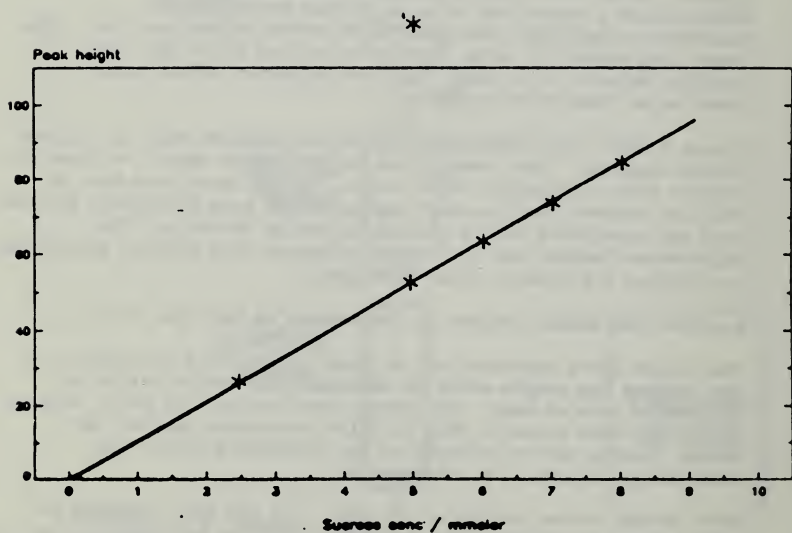


Figure 3.--Sucrose calibration curve for the enzymatic FIA system.

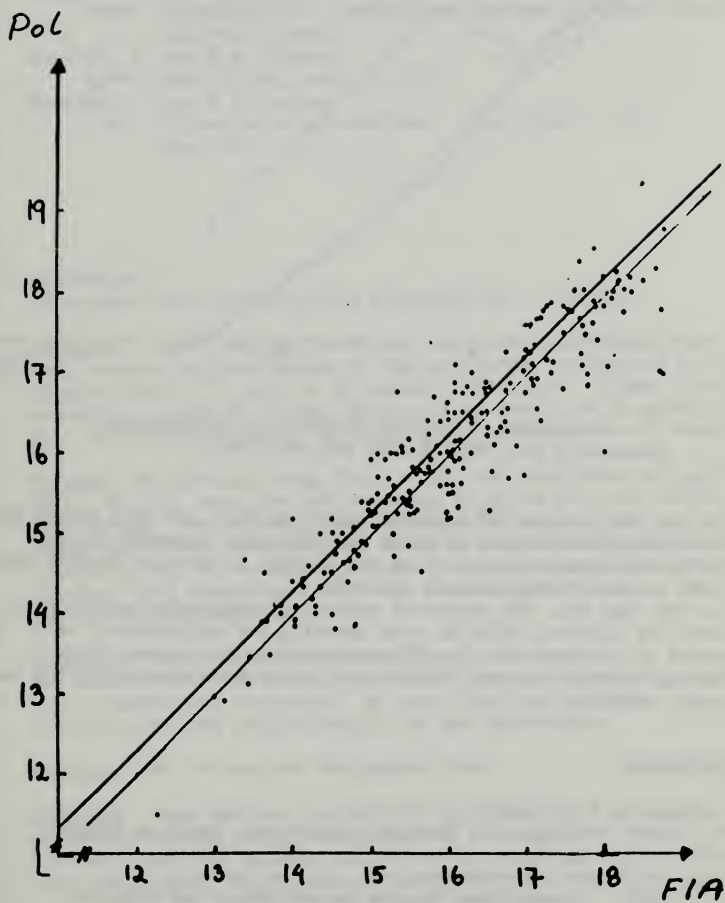


Figure 4.--Comparison of sucrose analysis between FIA and pol for the 1987 campaign.

Brei inoculated with *Leuconostoc*  
Comparison Polarimetry and FIA sucrose

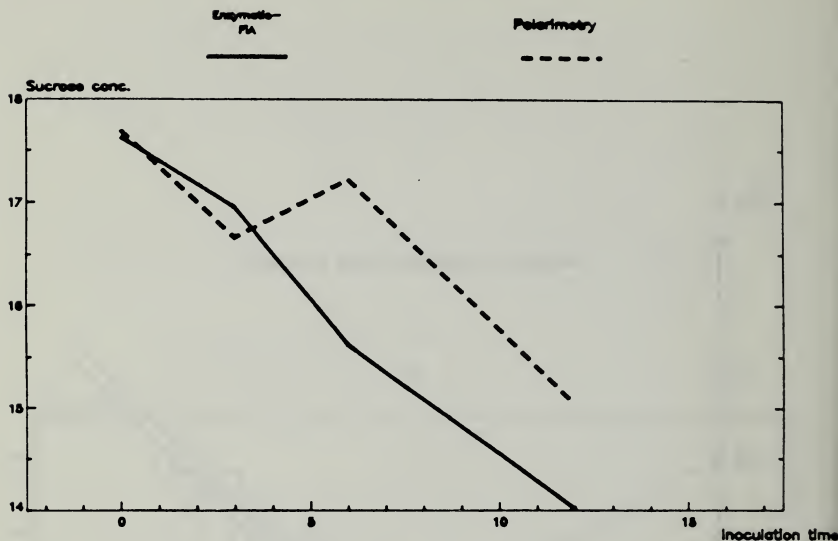


Figure 5.--Comparison of sucrose analysis of *Leuconostoc*-inoculated brei done by FIA and polarimetry.

In the early stages of implementation, we will use this system only for giving advice, but we think that the mere presence of this method will be an incentive to the growers to be more careful with beet storage during periods of frost.

In the long run, the enzymatic sucrose determination method will have its greatest value in seed breeding and agricultural work. It could be important in the effort to develop the optimal beet, having no doubt a great influence on the future economy of the beet sugar industry.

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#### DISCUSSION

(The paper and discussion were presented by J. Tjebbes.)

Question: Thank you very much for that very clear explanation of a very interesting development in the analytical technology for frost damaged beets. It will be of interest, I'm sure, to many other companies. I wonder if you could tell us how it is proposed to make this technology available to other sugar companies.

Tjebbes: We have not come that far yet and thus have nothing to sell. We will need the 1988 campaign to prove that the method is good enough. And I really don't think that we want to commercialize our development. We will rather use the ordinary scientific way of exchanging results. In this project we work together with researchers from the Lund University and we will publish jointly with them.

Question: Have you run determinations on the amount, if any, of the dextran that you precipitate out with aluminum sulfate during your standard pol procedure? We know that lead acetate precipitates some dextran during the pol measurement.

Tjebbes: No, we have not determined that.

Question: Have you ever considered splitting the two sample streams--in other words, analyzing the total glucose content and also analyzing the glucose content as is to get an estimation of both the sucrose content and the invert present.

Tjebbes: Yes, that is how we started out. We have however found that the precision in the sucrose determination is much superior with the described arrangement. For special purposes we can run glucose determinations separately.

Question: Your system has a quite sophisticated immobilized enzyme column. I noticed that you need to change them once a week, due to the death of the enzyme, I presume. I would guess that the system would need to have the columns changed much more often if it were used on cane juice, particularly because there would be a lot more polyphenols in the cane juice.

Tiebbes: I might guess the same, but, of course, we have no evidence to support it.

Question: Getting back to the earlier question about the availability of the instrument. When we used flow injection analysis before (for lactic acid), we had a difficult time with the source of the enzyme. I assume you are immobilizing your own enzyme, if not producing your own enzyme, and the concern in the future is will those be available? Not all of us are set up to produce these sorts of things.

Tiebbes: The individual enzymes are all easily available, but much of our success depends on the technique to immobilize them in the same reactor. This knowledge and technique is rather special and we might consider to commercialize it.

## HPLC TECHNIQUES IN PROCESS CONTROL IN SUGAR FACTORIES

W. S. Charles Tsang, M. A. Clarke and M. M. Valdes

Sugar Processing Research, Inc.

### INTRODUCTION

During the last decade, many articles have been published on the separation of carbohydrate mixtures by liquid chromatography (Abeydeera, 1983; Clarke and Tsang, 1983; Wnukowski, 1983; Rajakyla and Paloposki, 1983; Tsang and Clarke, 1984; Chorn and Hugo, 1984; Rajakyla, 1986; Tsang et al., 1986; Tsang et al., 1987). For example, at the S.I.T. 42nd Annual Meeting, we presented a paper describing an HPLC system dedicated to sugar analysis in factories and refineries (Clarke and Tsang, 1983). As a result of such research efforts, the HPLC separation and identification of sugar mixtures has become an accepted technique in the sugar industry (Ho, 1984; Hutton, 1984). Many uses were outlined in a recent review (Clarke, 1985).

The first part of this paper will relate results obtained from recent factory studies, on juices and process samples. The paper will also describe HPLC methods developed recently for analysis of trisaccharides such as kestoses and raffinose found in sugar samples.

### FACTORY STUDIES

#### Applications of HPLC to process control

A new HPLC model, Sugar Analyzer II (Waters Associates) was set up and tested in the control laboratory of a cane sugar factory (S.P.R.I. sponsoring company) for one week. Samples of juice across the mill, the clarifier and the evaporators were analyzed for sucrose and invert content. Wash water from bagasse and filter cake, waste water and effluent, were also monitored for sugar loss.

Two HPLC procedures were used: 1) a new Sugar Pak II column (ion exchange column in calcium form) for sucrose, glucose and fructose analysis (18 min) and 2) a Resolve C18 Radial Pak cartridge for rapid analysis (7-8 min) of sucrose and total invert.

### 1) Sucrose and Invert Levels in Juice

Among factory applications of HPLC analysis, determination of sucrose and invert levels in juices can give much useful information about sucrose levels entering the factory, and therefore about cane condition and mill performance. Data shown in Table 1 on crusher, mixed, and last mill juices are all examples. HPLC results are converted to "I on solids" basis, in order to compare juices of different Brix (To convert the wt/volume I from HPLC analysis: wt/volume I + (g/100 ml) equivalent to Brix of whole sample. The g/100 ml figure is obtained from Table 16, Cane Sugar Handbook, 11th edition).

Table 1.--HPLC analysis of cane juice.

Juice Sample	On volume, HPLC		Brix, wt. I (g/100 ml)	Pol	On solids, HPLC	
	I invert	I sucrose			I Invert	I Sucrose
Crusher	0.59	14.50	15.94 (16.90)	13.75	3.49	85.80
Mixed	0.60	12.94	14.20 (15.20)	12.03	3.95	85.13
Last Mill	0.55	2.93	3.66 (3.70)	3.01	14.86	79.19
Clarified	0.50	13.46	15.07 (16.00)	12.94	3.13	84.13
Syrup	3.33	64.00	57.64 (72.30)	49.40	4.61	88.52

### 2. Invert Formation and Removal in Clarifier.

The formation of invert in the clarifier is a potential pathway for sucrose loss. Comparison of sucrose and invert levels by HPLC on juice and clarified juice can show if total sugars are lost over clarification, and can be used to compare performance of different clarifier, and to compare different clarifier conditions for optimization of pH, temperature and other variables. Data in Table 1 again provide an example.

### 3. Invert Formation in Evaporators.

Comparison of invert levels on a solids basis in clarified juice entering evaporators, and in syrup exiting, can show up any loss of sucrose caused by inversion in the evaporation process. Some factories prefer to run evaporators on juice at

relatively low pH to minimize scale formation in the evaporators. Analysis, such as these shown in Table 1, can be used to observe invert formation: in the example shown, 1.48% of invert on solids, has formed across evaporation. The cost of this sucrose loss may then be weighed against the cost of increased evaporator scale formation at a slightly higher juice pH.

#### 4. True Sucrose in Bagasse and Mud.

HPLC analyses of bagasse wash (Figure 1a) filter muds (Figure 1b) and effluents can add valuable data to the calculation of a sucrose balance and factory losses. A pol on bagasse or filter mud wash will not show the identity of the polarizing compounds so that it is not known if this pol represents sucrose or glucose or dextran.

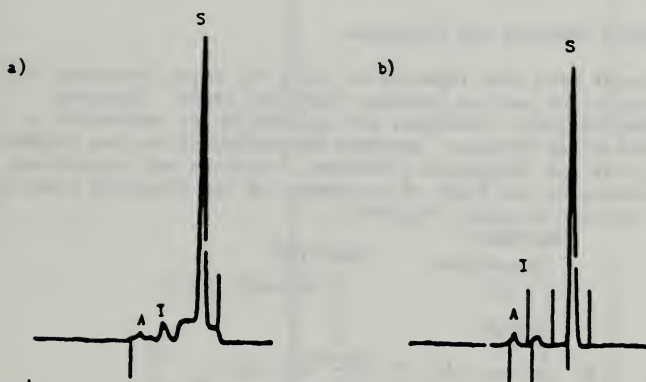


Figure 1.--Determination of sugars in (a) bagasse wash, (b) filter mud. Peak identities: A, ash; I, invert; S, sucrose.

#### 5. Trace analysis of factory water.

In waste water samples, a colorimetric carbohydrate test might show a negative or positive result, but could not show, as HPLC could, whether this was caused by actual sucrose loss or by



trace polysaccharide contamination. The improvement in sensitivity of the new Waters 410 refractive index detector makes it possible to detect trace amounts of sugar down to 1ppm. Figure 2 shows an analysis of condenser water. Trace amounts of both sucrose (3 ppm) and glucose (13 ppm) were found. There was almost no fructose present.

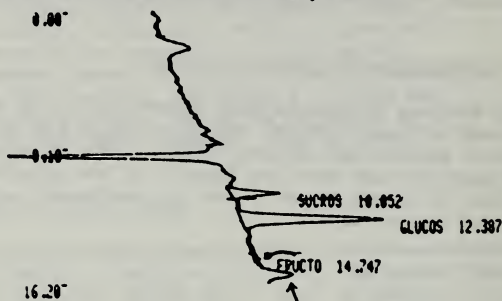


Figure 2.--Traces of sucrose and glucose in condenser water.

#### KESTOSE ANALYSIS AND SYNTHESIS

Kestoses have been reported as being the major trisaccharides in sugarcane and its products (Binkley, 1965). Improved chromatographic techniques now provide better separation of kestoses and nystose. Kestoses are fructosyl sucrose compounds and they are designated 1-kestose, 6-kestose and neo-kestose depending on the point of attachment of the fructosyl group to the sucrose molecule (Figure 3).

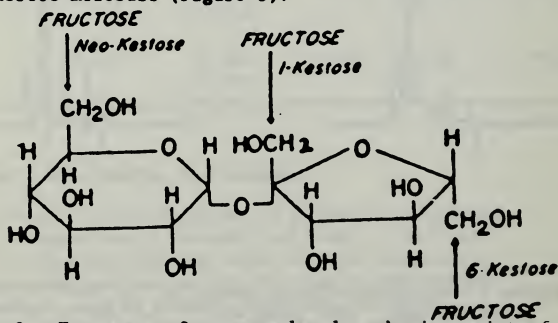


Figure 3.--Kestoses. Sucrose molecule, showing point of attachment of fructose to form the three known kestoses.

During an investigation of the stability of sucrose in diluted cane juice and raw sugar samples, the formation of an oligosaccharide had been observed. Attempts to synthesize the various kestoses for identification purposes have led to the

selection of an enzyme which gives a high yield of fructooligosaccharides.

1. Kestoses formation in cane juice.

A 10% cane juice sample was filtered with 0.45 $\mu$  Millipore membrane filter was allowed to store at ambient temperature for the week. The progress of sucrose degradation was followed by HPLC technique. A trisaccharide peak was observed after two days (Figure 4).

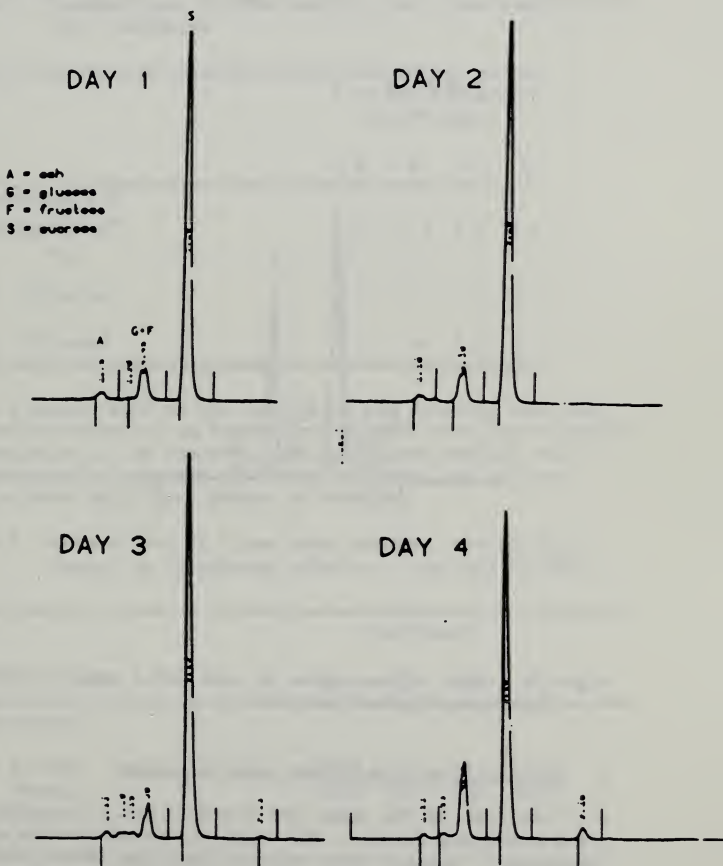


Figure 4.--HPLC chromatograms of a dilute cane juice sample which had been filtered with a 0.45 $\mu$  Millipore membrane filter.

By comparison, a different HPLC profile was observed at day 4 for a sample of the same juice which had not undergone the membrane filtration step. The juice sample became cloudy, probably due to the formation of dextran, and to bacterial growth. No trisaccharide peak was detected (Figure 5).

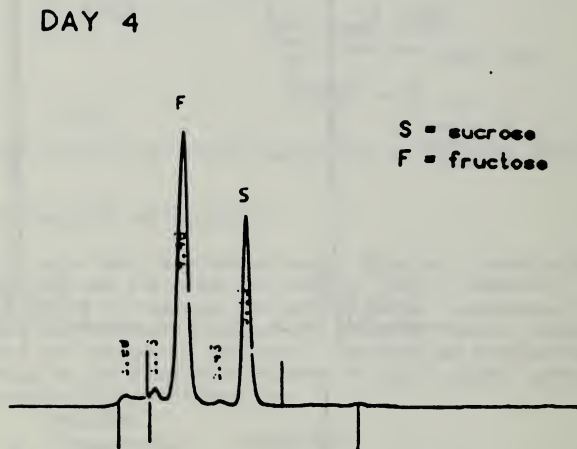


Figure 5.--HPLC chromatogram of cane juice sample without Millipore filtration.

## 2. Kestose formation in raw sugar solution.

A 1% solution of raw sugar was prepared and filtered with a 0.45 $\mu$  Millipore filter. The solution was maintained at ambient temperature; samples were injected into the HPLC systems on a daily basis. In two days, a trisaccharide was formed in addition to the invert. After the third day, the level of the trisaccharide remains fairly constant (12-13%) while the level

of invert increases (Figure 6). In a week the sucrose peak almost disappears. Invert (82%) and a trisaccharide (13%) were found as the major degradation products.

The amount of hestose formed in 4 different raw sugar samples during storage are analyzed and compared. After 2 days, a small amount of kestose is found in all samples except those which had not been filtered (Table 2).

Table 2.--Composition, by HPLC analysis, of 4 raw sugars stored in 1% solution.

		% of kestose formed in raw sugar			
		A	B	C	D
Day 3	Filtered	tr	1.9	2.1	1.0
	Not	--	--	--	--
4	Filtered	tr	2.9	3.2	2.4
5	Filtered	1	4.9	7.0	3.8

After 4 weeks, most of the sucrose in the filtered raw sugar samples is converted to invert and kestoses with the exception of raw sugar A. By contrast, the unfiltered samples still contain a lot of sucrose (Table 3) and three out of four samples have only trace amount of kestose.

Table 3.--Composition of 4 raw sugar samples, stored for 4 weeks, in 1% aqueous solution. Analysis by HPLC.

		RAW SUGAR			
		A	B	C	D
1.	Filtered				
	% Sucrose	58.4	--	--	4.0
	Invert	36.0	92.5	100	86.6
	Kestose	5.6	7.5	--	9.4
2.	Unfiltered				
	% Sucrose	51.2	62.6	86.8	47.6
	Invert	48.8	37.4	13.2	42.8
	Kestose	tr	tr	tr	8.6

Raw Sugar (1% solution)

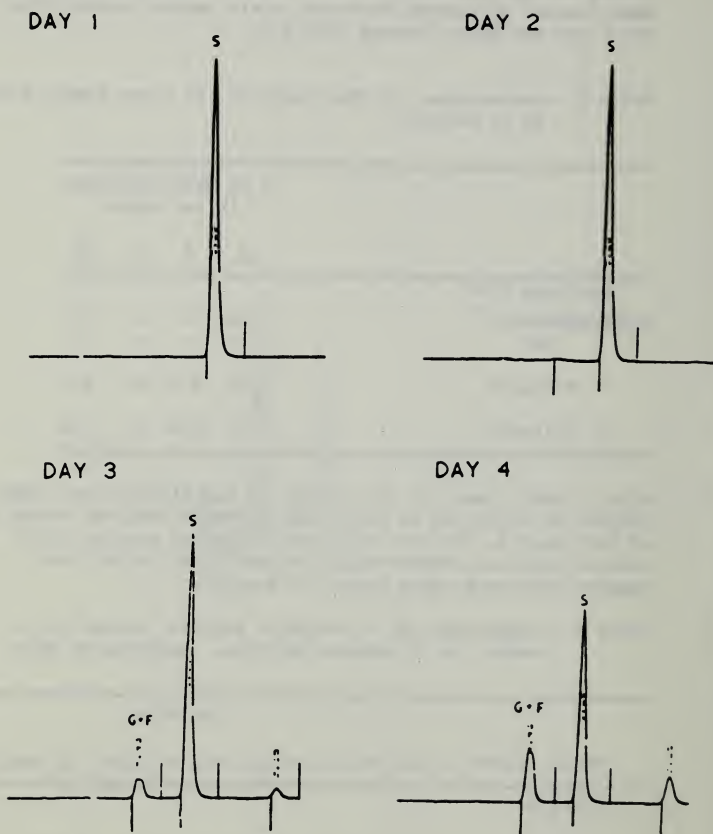


Figure 6.--Chromatograms of a 1% raw sugar sample purified through Millipore membrane filtration.



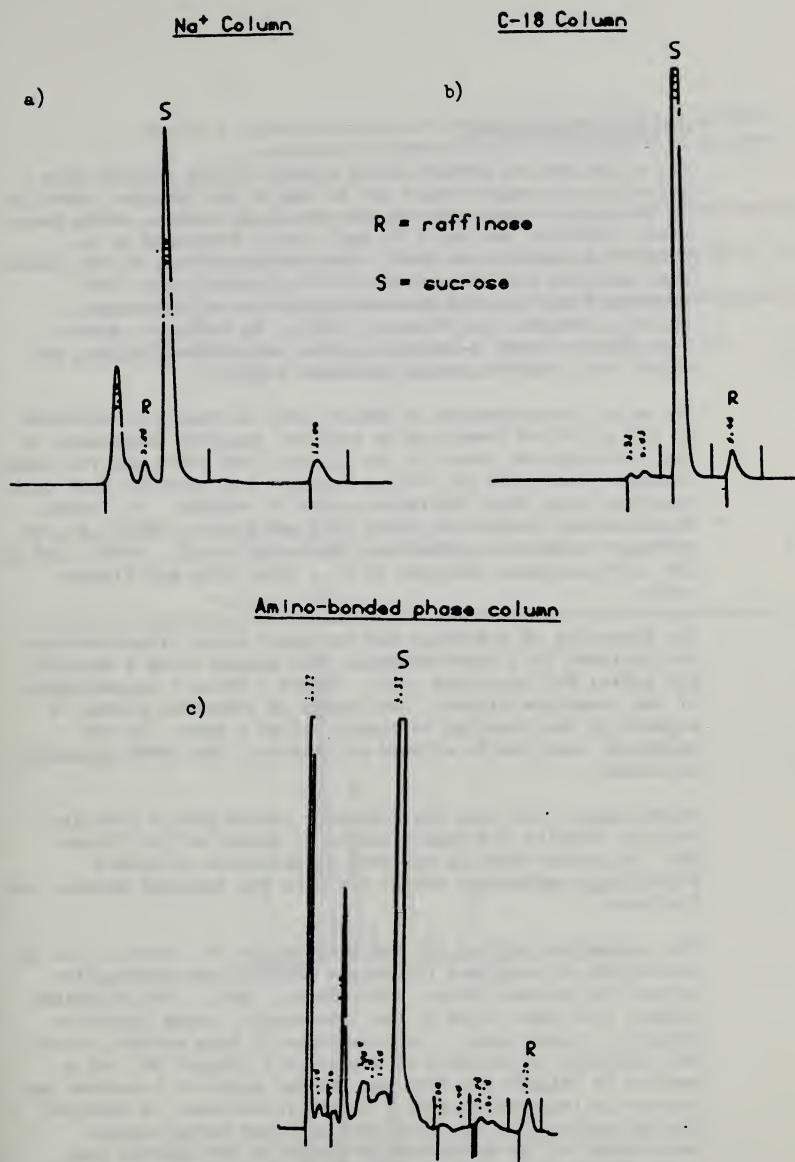


Figure 9.--HPLC chromatograms of beet molasses using three different columns. Peaks: R = raffinose; S = sucrose.

### Synthesis of kestoses

The trisaccharide formed during storage of the diluted cane juice and raw sugar sample may be one of the kestoses known to be produced by enzymatic action on sucrose (Gross, 1962; Pazur, 1962; Schaffler and Morel Du Boil, 1972; Straathof et al., 1986; Ivin and Clarke, 1987). In recent studies, it was found that purified sucrose-sucrose-fructosyltransferase (SST) produces 1-kestose and is essentially free of invertase activity (Wagener and Wiemken, 1987). In contrast, yeast invertase produces 6-kestoses as the main trisaccharide, and shows both transferase and hydrolase activity.

The major trisaccharide, 6-kestose (8%, in Figure 7) obtained by the action of invertase on sucrose, does not correspond to the trisaccharide found in the diluted cane juice and raw sugar sample. Separation of various kestoses and nystoses have been reported using three different types of columns: 1) amino-bonded silica (Schiweck, 1982; Ivin and Clarke, 1987), 2) ion exchange resin in calcium form (Straathof et al., 1986), and 3) C18 reversed-phase (Heyraud et al., 1984; Ivin and Clarke, 1987).

The formation of 6-kestose and two other minor trisaccharides was followed by a reversed-phase HPLC method using a Resolve C18 Radial Pak cartridge (5 $\mu$ ). Figure 7 shows a chromatogram of the invertase digest. The amount of 6-kestose formed is highest if the reaction is stopped after 1 hour. If the enzymatic reaction is allowed to continue, the level gradually decreases.

Surprisingly, the peak for 6-kestose eluted before that for sucrose, despite the higher molecular weight of the former. The C18 column normally resolves carbohydrate oligomers according to molecular weight and does not separate glucose and fructose.

The traditional method for the preparation of 1-kestose was by the action of  $\alpha$ -amylase (an enzyme mixture from *Aspergillus oryzae*) on sucrose (Gross, 1962; Pazur, 1962). Two  $\alpha$ -amylase enzymes have been tried in our laboratory: crude fungal  $\alpha$ -amylase I (Sigma Corp.), and  $\alpha$ -amylase II from another source. HPLC analysis of products of  $\alpha$ -amylase I (Figure 8a) and  $\alpha$ -amylase II (Figure 8b) show that higher yield of 1-kestose and nystose (a tetrasaccharide, a fructosyl-kestose) is obtained from  $\alpha$ -amylase II. The 1-kestose produced enzymatically corresponds to the trisaccharide found in the diluted cane juice and raw sugar sample. The ratio  $r_h/r_n$ , where  $r_h$  is the initial rate of formation of kestose and  $R_n$  is the initial rate of hydrolysis, for  $\alpha$ -amylase I (Table 4). As a result, higher yields of total fructooligosaccharides (~50%) are obtained.

Table 4.--Relative proportion of oligosaccharide mixture obtained by the action of  $\alpha$ -amylase on sucrose.

	24 hrs	Invert	Sucrose	1-Kest	6-Kest	neo-Kest	Nyst.
$\alpha$ -amylase I	I (by wt)	49.80	18.74	23.91	3.22	1.00	3.48
	mM	276.70	54.80	47.44	8.37		5.74

$$r_k/r_h = 0.6$$

$\alpha$ -amylase II	I (by wt)	21.54	30.93	38.74	0.83	1.44	6.5
	mM	119.70	90.73	76.87	4.50		9.76

$$r_k/r_h = 10$$

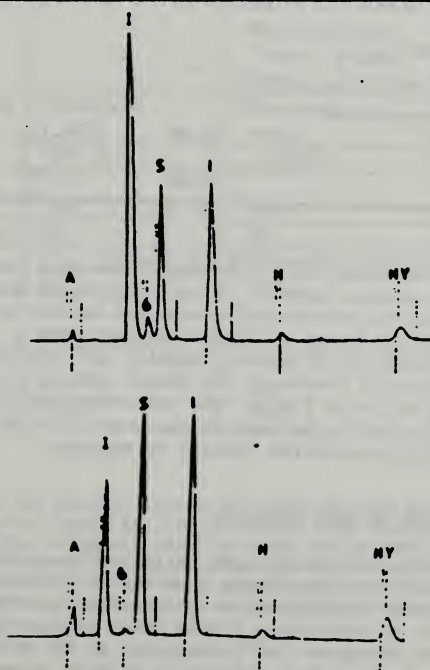


Figure 8.--Separation of sugars on a Resolve C18 Radial Pak cartridge of (a)  $\alpha$ -amylase I digest, and (b)  $\alpha$ -amylase II digest. Peaks: A= ash; I = invert; S = sucrose; 6 = 6-kestose; l = 1-kestose; N = neo-kestose; NY = nystose.

The high transfructosylating and low hydrolyzing activities processed by  $\alpha$ -amylase II (*A. oryzae*) compare favorably with those reported recently for *A. niger* (Hidaka et al., 1988) and *A. phoenicis* (van Balken et al., 1988) (Table 5).

Table 5.--Production of oligosaccharides from different microorganisms.

	Organism			
	<i>A. niger</i>	<i>A. phoenicis</i>	<i>A. oryzae</i>	
Incubation time (hr)	72	18	32	48
1-kestose (%)	17.30	38.14	37.65	35.80
nystose (%)	32.00	18.00	8.34	13.61
fructofuranosyl-nystose (%)	7.20	--	--	--
6- and neo-kestose	--	--	2.74	3.48
% oligosaccharide of total	56.60	56.14	48.73	52.89
$r_K/r_H$	12.10	4.70	5.00	4.00

A new sweetener, called Neosugar, has been developed recently (Hidaka et al., 1988). It is a mixture of 1-kestose, nystose and 1 $\beta$ -fructofuranosylnystose and manufactured commercially from sucrose using an enzyme obtained from *A. niger* ATCC 20611. It was found to be non-cariogenic, and reported to be a nondigestible sweetener for humans (Oku et al., 1984). The results in Table 5 show that the  $\alpha$ -amylase II (from *A. oryzae*) has potential to be used in the production of fructooligosaccharide similar to Neosugar.

#### RAFFINOSE IN BEET MOLASSES

Raffinose, a trisaccharide (galactosylsucrose), is present in sugarbeets in significant quantities, particularly in beets which have been subjected to prolonged, cold temperature. Since it is chemically stable under processing conditions, it does not decompose but accumulates in the molasses. Practically, it is important to be able to measure the

raffinose level for better crystallization and higher recovery in the sugar end.

The level of raffinose in beet molasses had been determined by HPLC using amino-bonded silica columns (Schiveck, 1982; Reinfeld et al., 1988). In this study, three different HPLC columns are used for the determination of raffinose content in a series of beet molasses. The results are compared with those obtained from the enzymatic method--a kit from Boehringer Co. Table 6 shows the conditions of HPLC analysis for beet molasses.

Table 6.--HPLC experimental details.

	Ion Exchange	Reverse-phase	Amino-bonded phase
Column	Resolution Na <sup>+</sup> , 300 x 7.8 mm	Waters Resolve C18 5µ Radial Pak Cartridge	Bio-S11 Amino SS 250 x 4.6 mm
Guard Column	---	Resolve C18 Guard- Pak precolumn Insert; BioRad deashing cartridge	BioRad Micro- guard Bio-S11 Amino SS
Mobile phase	Deionized water	Deionized water	Acetonitrile/ water (75:25)
Temp.	85° C	Ambient	35° C
Flow rate (ml/min)	0.5	0.8	1.2
Sample conc.	2%	2%	10%
Detector	Waters 410 Differential Refractometer		
Sensitivity	64	128	28
Time for Analysis	13	12	20
Injection Volume (ul)	20	20	20
CV (%, n=8)	1.23	1.07	1.38

In the ion exchange system, all three kestoses co-elute with raffinose (Figure 9a) and as a result, the reading for raffinose is highest among the other system (Table 7). In the reversed-phase system, only the 1-kestose co-elutes with raffinose (Figure 9b) which explains why the 1 raffinose is not as high as the result from the ion exchange system.



Table 7.--Raffinose (I) determined by HPLC and enzymatic method.

Beet Molasses Samples	HPLC ( Unclarified samples )			Enzymatic Method ( Clarified )
	Ion Exchange	Reverse-Phase	Amino-bonded Phase	
1	2.37	2.00	1.43	1.19
2	0.85	0.73	0.39	0.36
3	2.00	2.44	1.84	1.72
4	2.25	2.28	1.91	1.50
5	2.29	1.66	1.22	0.98
6	1.16	1.18	0.67	0.62
7	1.07	0.82	0.46	0.56
8	1.06	1.48	1.16	1.13
9	1.51	1.48	1.10	1.15
10	1.64	1.40	0.67	0.74
11	1.09	1.09	0.73	0.69
12	1.14	1.10	0.80	0.84

Both the ion exchange and the reverse phase systems, however, have the advantages that they use aqueous solvents and regenerable columns and the run time is relatively short. The analysis is most suitable for samples with other trisaccharides at minimal levels.

The amino-bonded phase silica system employs acetonitrile-water as the mobile phase and a non-regenerable column, but has been shown (Schlueck, 1982) to give a specific analysis for raffinose. In this study, it gives the most accurate analysis for raffinose among the three HPLC methods because it is possible to separate various kestoses, and galactinol from raffinose.

Enzymatic methods: The HPLC raffinose results were compared with those obtained by enzymatic methods. The enzymatic kit (Boehringer Mannheim) is used for the determination of raffinose in beet molasses.

In the enzymatic analysis, other substances, e.g. galactinol, which are present in beet molasses, can be substrates for  $\alpha$ -galactoside and cause a falsely high analysis. These can be removed by a lead subacetate clarification. The absorbance differences for a series of standard ranging from 0.1 to 1.2 g/liter were determined before starting the assay for the molasses sample.

Statistically, as shown in Table 8, there is no significant difference between the amino-bonded column HPLC method and the enzymatic method. A constant factor causes difference between the ion exchange and reverse phase values, and the amine HPLC and enzymatic analysis. This is explained by the fact that kestoses and/or galactinol co-elute with raffinose on the ion exchange and reverse-phase system.

Table 8.--Oneway analysis of variance of % raffinose determination (general linear models procedure).

WHEN  $P_r < 0.01$ , DIFFERENCE IS SIGNIFICANT AT 0.01.

CONTRAST	$P_r > F$
1) AMINE HPLC vs. ENZYME	0.6075
2) ION-EX. HPLC VS. (AMINE HPLC-ENZYM)	0.0001
3) REV. PHASE HPLC VS. (AMINE HPLC-ENZYM)	0.0004
4) (ION-EX. + REV. PHASE) HPLC VS. (AMINE HPLC-ENZYM)	0.0001

## SUMMARY

This paper has presented and discussed results from projects incorporating HPLC analysis in sugar factory process control.

The ability to observe true sucrose values in process is outlined, and examples are given from trials at a raw cane sugar factory.

Observations on trisaccharide formation in cane sugar are reported and related to formation of kestoses. These findings led to the identification of a new system to produce kestoses ("Neosugar") in high yield.

Three HPLC systems for determination of raffinose in beet molasses have been studied, and are compared with an enzymatic method.

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## DISCUSSION

Question: This is not a question but rather a comment. When you are looking at inversion in the front end of the factory, as you mentioned, you have to report the data on a solids basis because the brix is constantly changing. We found that we can simply report the glucose level or the invert level relative to sucrose. You would then have a glucose-sucrose ratio which gives you exactly the same information as by brix measurement, but you avoid doing the brix analysis and having to convert the grams per ml to grams, so it is a lot easier.

Tsang: Thank you.

Question: Perhaps the sugar industry ought to hesitate to talk about Neo-sugar or to promote it too much. When you talk about its non-cariogenicity and reduced calorific value, you are undoing some very expensive medical lobbying activities of the past few years.

One surprising result to me was on the samples of juice that you kept for 4 weeks at room temperature: In the filtered juice the sucrose disappeared and in the unfiltered juice, the sucrose survived. How do you explain that?

Tsang: I think that the Millipore filtration must take out invertase or a kestose inhibitor.

Question: But the sucrose has disappeared in the filtered solution. Where has it gone?

Tsang: About 85% of it has gone to invert and about 13% to kestose.

Question: And so it goes faster after you have filtered it, after you have removed micro-organisms or enzymes. I think possibly you have a feedback mechanism. When you don't filter, there are organisms that, after it has gone to kestose, bring it back to sucrose.

Tsang: That is a possibility.

## **NEW TECHNIQUES FOR MONITORING PERFORMANCE IN CANESORB<sup>R</sup> SYSTEMS**

**Bruce D. Wells and D. A. Leister**

**Calgon Carbon Corporation**

### **INTRODUCTION**

Calgon Carbon Corporation's Canesorb<sup>R</sup> process combines granular activated carbon with bone char as an admixture to attain a new cane sugar decolorizing system. This use of granular activated carbon with bone char has provided a balanced decolorization and de-ashing system in many refineries, worldwide. The blended adsorbent is commonly made up of about 20 weight percent Canesorb granular activated carbon and 80 weight percent bone char. The process utilizes existing bone char reactivation equipment - pipe kilns and multiple hearth furnaces. The Canesorb blend is reactivated in its blended in-use form - that is, there is no attempt to separate the mixture into its components - carbon and char - prior to reactivation.

When changes in process conditions are made in any portion of the refinery, the impact of these changes must be measured relative to long term performance of the decolorizing station.

Each Canesorb system contains several thousand cubic feet of adsorbent. This large inventory of adsorbent creates an inertia within the system that, in general, is deemed beneficial. That is, it makes the system more tolerant of upsets. However, this inertia is also a problem, in that, the results from any upstream process changes may not be seen for several weeks or months. Consequently, it is very difficult to monitor the effects of process changes on the Canesorb adsorbent performance with conventional testing procedures.

Two methods of monitoring the condition of the Canesorb blend will be discussed. In addition, the impact of changes in process conditions on adsorbent decolorizing properties and pore structure will be described. The general refinery decolorization station performance improvement will be related to the improved adsorbent properties.

## APPROACH TO MONITORING

It is very important to be able to monitor the impacts, both favorable and unfavorable, of any changes in the process conditions, in an accurate and timely fashion. With this in mind, Calgon Carbon Corporation has utilized two laboratory techniques to help evaluate process changes by monitoring the quality of the reactivated adsorbent blend.

The first test is a Relative Efficiency Test. The Relative Efficiency Test compares the decolorizing ability of the reactivated adsorbent to that of a virgin blended material. This test simulates the process by directly measuring decolorizing ability of the service blend of Canesorb and bonechar.

The Relative Efficiency Test utilized by Calgon Carbon Corporation was adopted from the procedure in use at Lantic Sugar Refinery, St. John, N.B., Canada. It can be used with the unseparated Canesorb blend or with either separated component. However, Calgon Carbon Corporation normally reports data for the unseparated blend. The test is performed at two levels of contact time between the adsorbent and test liquor - one hour and four hours. The one hour test is rate related, while the four-hour test is total capacity related.

The second test method is an instrumental analysis technique which measures the pore size distribution of the reactivated adsorbent and compares it to that of the virgin blend. This procedure can indicate if pore volume is increasing, decreasing or maintaining status quo. In addition, loss, or gain, of surface area within a particular range of pore diameters can be monitored.

These two techniques now give Calgon Carbon Corporation and Canesorb users a practical measurement of the blended adsorbent decolorizing performance and a "scientific" means of understanding the impact of process changes on the adsorbent pore structure. The experience of a year and a half has shown the trends exhibited by the two tests can be used to successfully monitor the Canesorb performance. That is, the results of both tests tend to parallel the actual plant decolorization system performance trends.

The response to improved reactivation of the Canesorb blend will be followed from the actual plant decolorization standpoint as well. It will be shown that actual plant decolorization performance can be projected by the Relative Efficiency test results. It is possible to correlate improvements in Relative Efficiency and changes in pore size distribution with improved plant decolorization performance.

#### BASIC ADSORPTION THEORY

Activated carbon possesses very high internal pore area - over 1000 m<sup>2</sup>/gm. The very high surface area of these very small pores is responsible for activated carbon's decolorization ability. The adsorbed color bodies are held to these internal surfaces by Van der Waals forces. The color bodies are attracted to highly energized micropores and held very strongly by the surface energy dependent forces. Although both activated carbon and bone char are microporous adsorbents, they are very different from each other in terms of internal pore structure. Activated carbon pores can be classified as: macropores, mesopores, and micropores. Figure 1 is an artist's rendition illustrating the pore structure of activated carbon. The macropores, or large diameter pores, can measure up to thousands of Angstroms in diameter. The mesopores, or transition pores, are a few hundred Angstroms in diameter. The micropores, or storage pores, measure approximately 20 angstroms or less in diameter.

Figures 2 and 3 are artists' depictions of a single pore in activated carbon and bone char, respectively. The relative absence of macro and micropore structure in bone char is the reason for the major difference in sugar decolorization ability between bone char and activated carbon.

Figures 4, 5, and 6 show the actual measured pore size distributions of virgin Canesorb alone, virgin bone char alone and the 80/20 admixture of virgin bonechar and Canesorb.

Adsorbents such as activated carbon are normally characterized by a number of different traditional chemical tests. For example, the molasses number is a decolorization value, for a standard molasses solution, and correlates with the adsorption rate for large molecules. Carbons with high molasses numbers tend to have a larger proportion of pores larger than 100 Angstroms. Another common characterization test is the iodine number. It measures a carbon's ability to remove iodine from a



# **CROSS SECTION VIEW OF HYPOTHETICAL PORE STRUCTURE**



Figure 1. Artist's rendition of hypothetical pore structure of activated carbon.



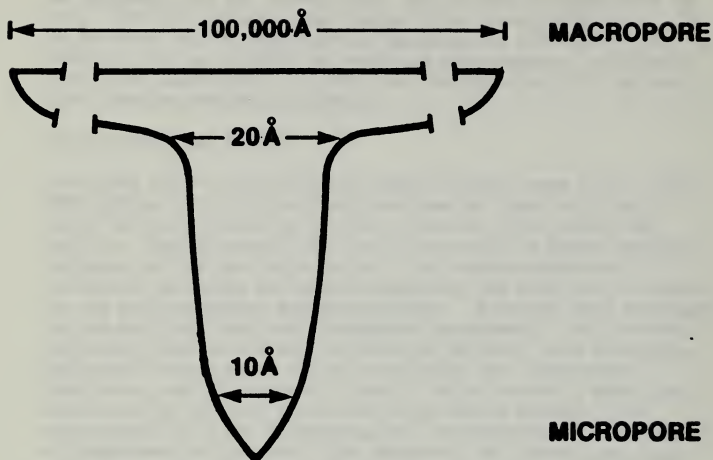


Figure 2. Hypothetical structure of a single activated carbon pore.

### BONE CHAR PORE

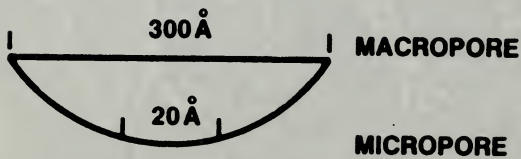


Figure 3. Hypothetical structure of a single bone char pore.

## CANESORB CARBON

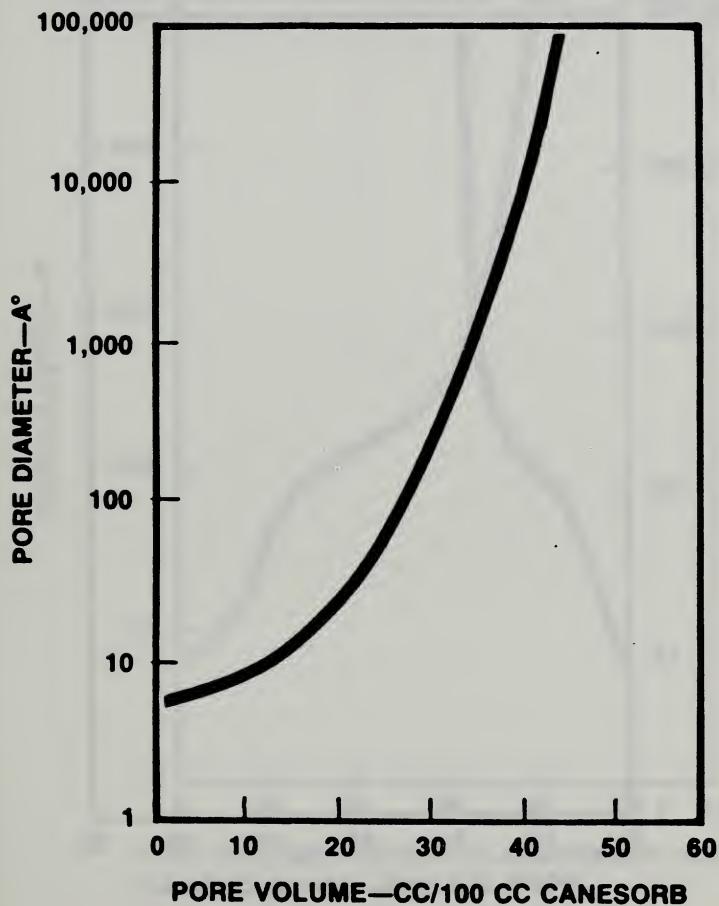


Figure 4. Experimentally measured pore size distribution of virgin Canesorb carbon alone.

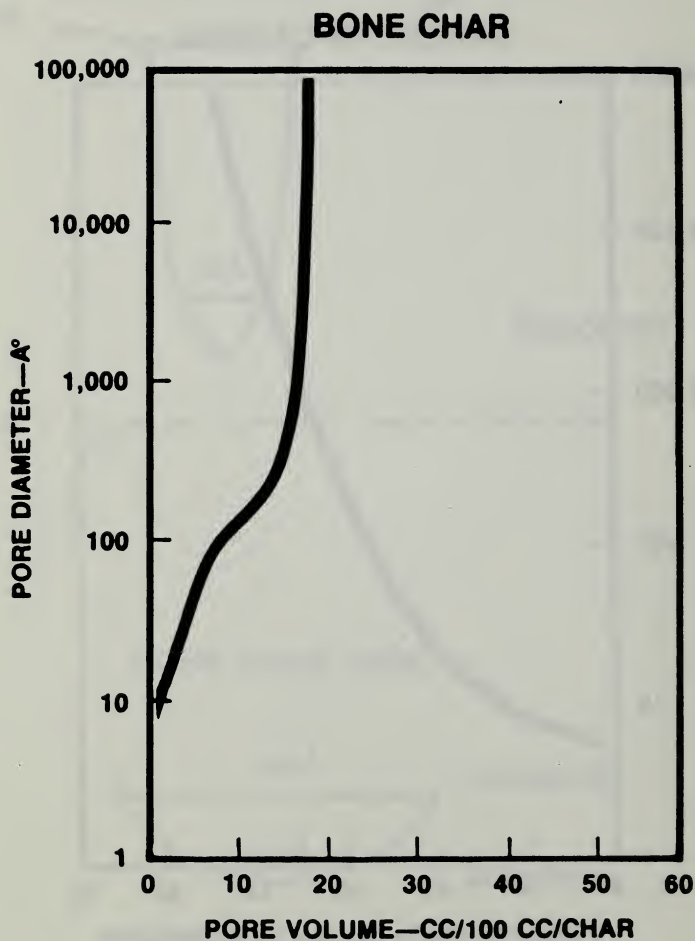


Figure 5. Experimentally measured pore size distribution of virgin bone char.

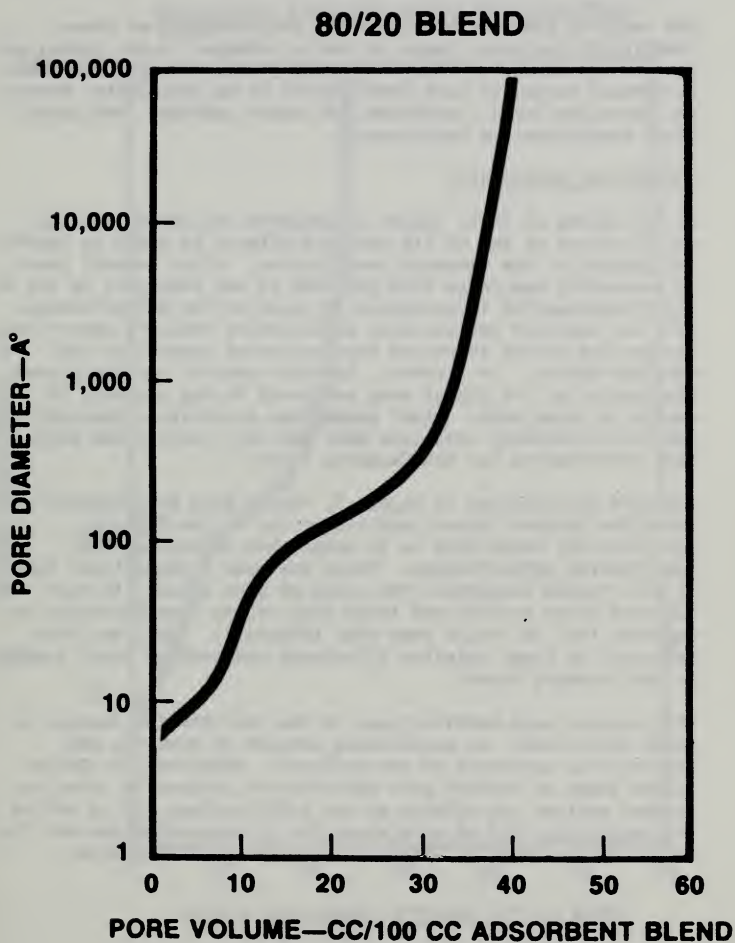


Figure 6. Experimentally measured pore size distribution of an 80/20 admixture of virgin bone char and virgin Canesorb carbon.

standard KI-iodine solution and correlates, roughly, with total surface area. It is a measure of capacity for smaller molecules. Many similar surrogate tests are used to attempt to characterize, or fingerprint, a given carbon.

The industry has been challenged by the inability of these traditional surrogate tests to monitor changes in the carbon and char in the Canesorb blend as upstream process changes were made. In several cases, we have found little or no correlation between the surrogate tests - molasses and iodine numbers - and actual plant decolorization performance.

#### A Practical Application

In the Spring of 1985, Calgon recommended certain furnace modifications at one of its Canesorb clients in order to improve the quality of the Canesorb reactivation. After several years of successful operation with Canesorb it was necessary to try to effect substantial improvements in reactivation effectiveness. This was required because many evolutionary refinery operational changes had placed increased decolorization demands on the Canesorb system. As a result, Canesorb quality had diminished. The results of the change were monitored during the next 18 months in three ways: plant production statistics, Canesorb Relative Efficiency test (one hour and four hours), and pore size distribution for the Canesorb blend.

The pore distribution in Figure 7, "March Pore Distribution", shows the Canesorb blend condition prior to the furnace modifications which were to be undertaken to improve the reactivation effectiveness. There has been a significant loss of pore volume throughout the range of pore sizes. In fact, the Canesorb blend profile and total pore volume were beginning to approach that of virgin bone char (Figure 5). This was also reflected in lower Relative Efficiency and reduced color loading on the Canesorb blend.

The customer made modifications to the reactivation furnace in early April 1985. An established program of sampling and analysis was continued by the refinery. Additionally, Calgon Carbon began to perform pore distribution studies in order to further monitor the effects of the modifications and to better understand the role of pore structure in decolorization and its impact on the Relative Efficiency of the blended material.



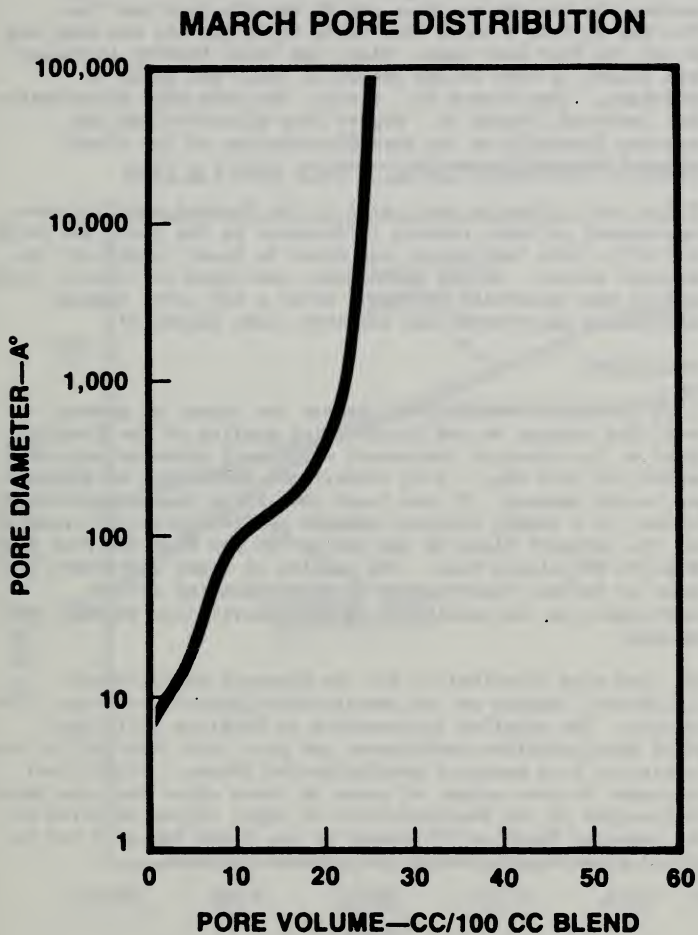


Figure 7. March pore distribution of Canesorb blend prior to furnace modifications.

The results of these studies were very encouraging. The Relative Efficiency of the blend began to improve within two weeks of the implementation of our recommended furnace modifications. This improvement continued over the next 18 months and, as shown in Figure 8, the Relative Efficiency reached 104 for the one hour and 98 for the four hour test. This was up from a March 1985 value of 88 for the one hour and 81 for the four hour test. Also, the color loading increased from 10,000 to over 13,500 pounds of color per pound of adsorbent, (See Figure 9). Lastly, the pore size distribution also improved (Figure 10 - August Pore Distribution) and compared favorably to the pore distribution of the virgin Canesorb blend (Figure 6).

Within about three to four weeks of the furnace modifications, improvement in color removal performance in the plant was noted. Initially, this improvement was noted in final colors off the Canesorb system. As the performance continued to improve, cycle lengths were gradually increased until a 23% color loading performance improvement was achieved, (see Figure 9).

#### CONCLUSIONS

It is difficult to accurately assess the impact of process condition changes on the decolorizing quality of the Canesorb blend or its separate components - Canesorb granular activated carbon and bone char - with traditional tests such as molasses and iodine numbers. It was found that it is indeed possible to follow, in a timely fashion, changes in decolorization capacity for the Canesorb blend by the use of the One Hour and Four Hour Relative Efficiency Test. The results of these tests have been shown to reflect improvements in decolorization system performance as the conditions in the reactivation furnace were changed.

The pore size distribution for the Canesorb blend showed significant changes as the reactivation furnace conditions were changed. The parallel improvements in Relative Efficiency, plant decolorization performance and pore size distribution were consistent with accepted decolorization theory. Significant increases in pore volume of pores of those sizes that are known to function in the decolorization of sugar liquor occurred as the measured Relative Efficiency of the blend improved and the actual plant decolorization performance improved.

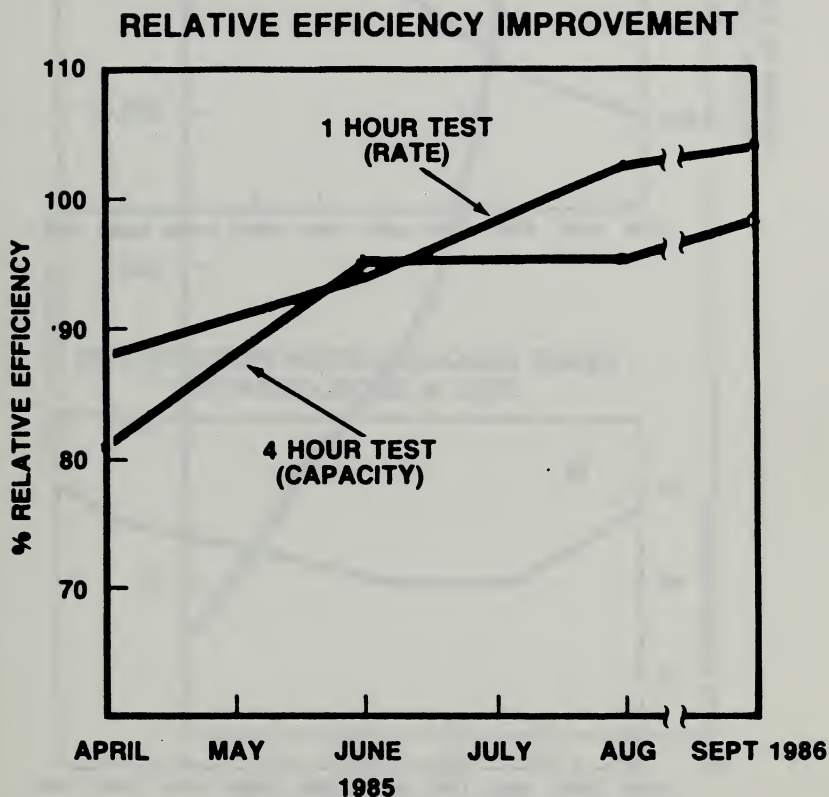
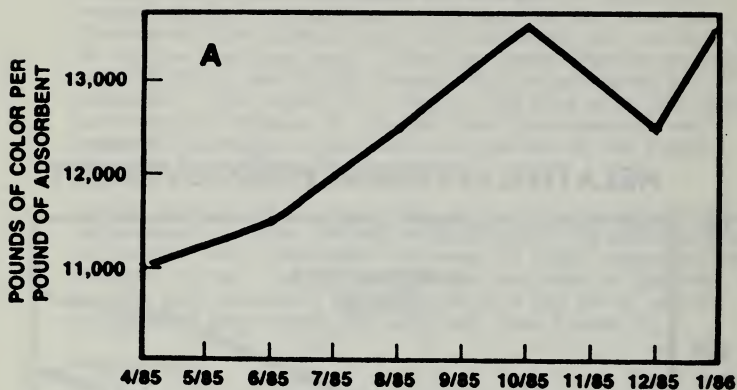


Figure 8. Relative Efficiency improvement of blend after furnace modifications.

# PLANT DECOLORIZATION PERFORMANCE COLOR LOADING



# PLANT DECOLORIZATION PERFORMANCE AVG. % DECOLORIZATION

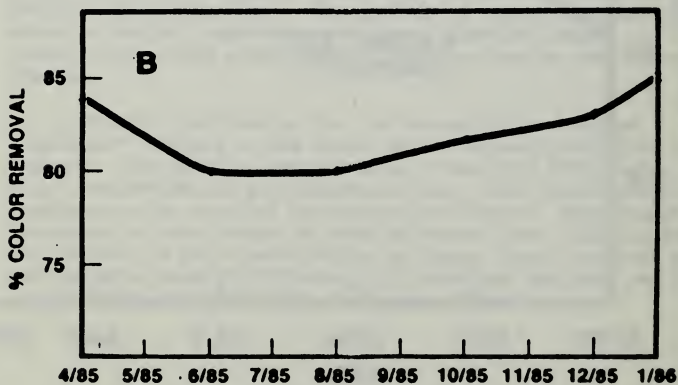


Figure 9. Improvement in plant decolorization performance after furnace modification. A: Color loading. B: Average percent decolorization.

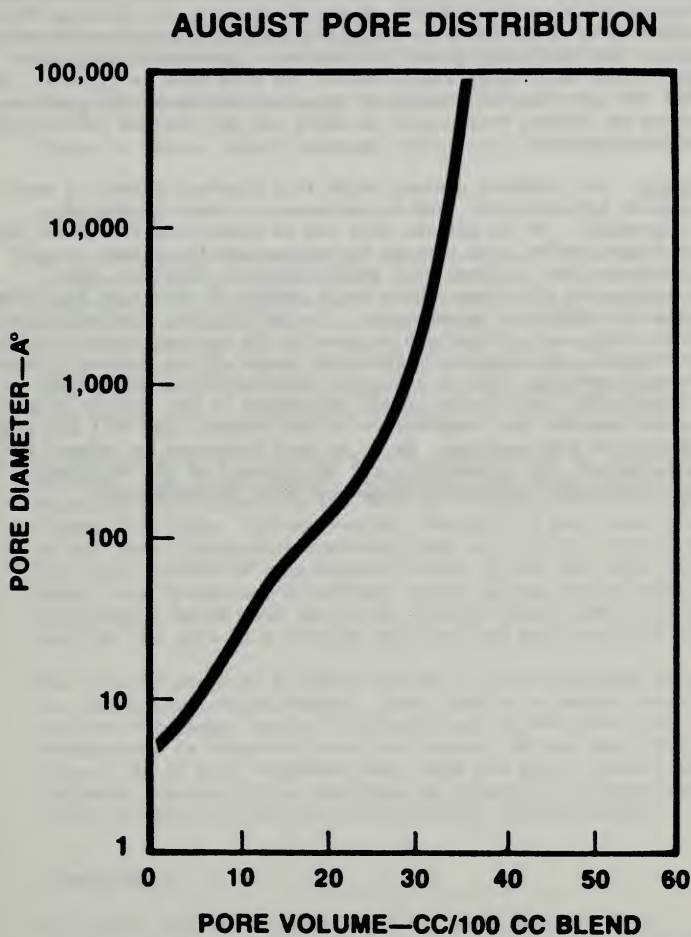


Figure 10. August pore distribution of Canesorb blend after furnace modifications. (Compare to Figure 7.)



## DISCUSSION

Question: I would like to introduce a question from a completely different field. In the gasification of char, which possibly has some similarities to the physical chemistry that you are dealing with, the reaction of char, in terms of gasification, is a surface reaction in pores with oxidizing gases. It reaches a pronounced maximum at particular char temperatures--450°C for wood char is typical. Do you see the same kind of thing with the decolorizing activity of char? Is there an optimum temperature at which you get maximum decolorizing effectiveness?

Wells: The Canesorb system, which is a blended system, is very complex to reactivate, and it represents a whole series of compromises. It is not the best set of conditions for either char or activated carbon, and that is why the gas-solid contact is very important. In a system with which I am more familiar--the reactivation of carbon--there are a number of reactions that take place at different temperatures. To get the best decolorization efficiency, it is important to provide the correct temperature and atmosphere relationship in different parts of the furnace. There are certain factors, such as water-gas relationships, which begin at 1450-1500°F, that if the proper atmosphere is not present when the carbon reaches that temperature in the furnace, you will not gain the benefit of the reaction. So it is very important to balance the temperature, the atmosphere, and the contact of the absorbent. These are really the only three things you have to manipulate.

## A CORRELATION BETWEEN COLOR REMOVAL (BATCH TEST) BY BONE CHARCOAL AND THE CORRESPONDING RATE OF WATER VAPOR ADSORPTION

Chung Chi Chou, Amstar Sugar Corporation, New York, NY 10020

Victor R. Deitz, Naval Research Laboratory, Washington, D.C. 20375

### INTRODUCTION

Revivified bone char begins to absorb oxygen and water vapor during transport from the kilns to the char filters. The settling operation, contacting the char with sugar liquor, is generally considered to complete the wetting of all particles of bone char with the settling liquor. The latter on the average is a washed sugar liquor (WSL) of 64 to 67 Brix between 170 to 180°F. It may take about 3 to 5 hours to complete the settling operation which is then followed by a continuous running of the liquor cycle.

A question may be raised as to the completeness of the above wetting process. The color removal mechanism takes place across the solution-solid interface and this is obviously more efficient for a larger interface. During current research on gas-solid adsorption at the Naval Research Laboratory, the rate of water vapor adsorption has been studied in some detail. Also, during the past several years, the Operations Laboratory of the Amstar Sugar Corporation has developed a batch test for color removal (Chou, 1983) and has applied the test on a routine basis to new and service stock chars.

The present paper is a report on the correlation found between these two independent measurements. Both involve a complex series of surface reactions, and it is likely that in each case the measurement is concerned with the slowest of the rate determining steps. It is also suggested that both the color removal capacity and water up-take of an adsorbent is generally characterized by the total surface area and pore structure of that adsorbent.

### EXPERIMENTAL

The batch test for color removal has been reported by C. C. Chou (1983). The test is based on a simplification of the classical isotherm equation developed by Langmuir and Freundlich. The rate of adsorption (I decolorization) of the new test is a measurement of the color removed by an adsorbent in one hour using 4 grams of bone char and 20 ml of 30 Brix reference liquor at a temperature of  $75 \pm 1.0^\circ\text{C}$ .

The weight increases of the samples were determined at regular intervals upon exposure to a humidified air flow of constant relative humidity and temperature. The weight increase is strongly dependent on the preliminary drying of the sample. Since drying in air above 100°C also results in losses due to oxidation, the samples were dried in nitrogen at 120°C and after cooling to room temperature, the nitrogen was displaced with dry air before the first weighing. Between exposure intervals, the sample tube was protected by T cap joints.

## RESULTS

Typical data are tabulated (Table 1) for a new bone char and a Brooklyn discard char (bulk density = 69 lbs/ft<sup>3</sup>). The results (Figure 1) show the large spread between a new bone char and discard char for the take-up of water vapor.

Table 1.--Water vapor pick-up by two samples of bone char.

Sequence	Total Time Interval (min.)	Total Weight Increase g.	Temp. °C	Relative Humidity % RH	Cumulative Weight Increase %
<b>New bone charcoal</b>					
Initial	0	0			
1	10	0.1791	23.4	79.9	2.452
2	22	0.2752	23.4	80.4	3.768
3	45	0.3609	23.4	78.3	4.941
4	72	0.4189	23.7	80.5	5.735
5	105	0.4632	23.9	80.5	6.341
6	145	0.4959	24.2	80.0	6.789
7	172	0.5100	24.2	77.8	6.982
<b>Brooklyn discard char</b>					
Initial	0	0			
1	10	0.0925	24.9	80.0	0.677
2	20	0.1202	24.9	80.6	0.880
3	31	0.1387	24.9	80.6	1.016
4	57	0.1599	25.0	80.0	1.171
5	96	0.1737	25.1	80.6	1.272
6	137	0.1807	25.3	80.6	1.323
7	180	0.1825	25.4	80.6	1.336

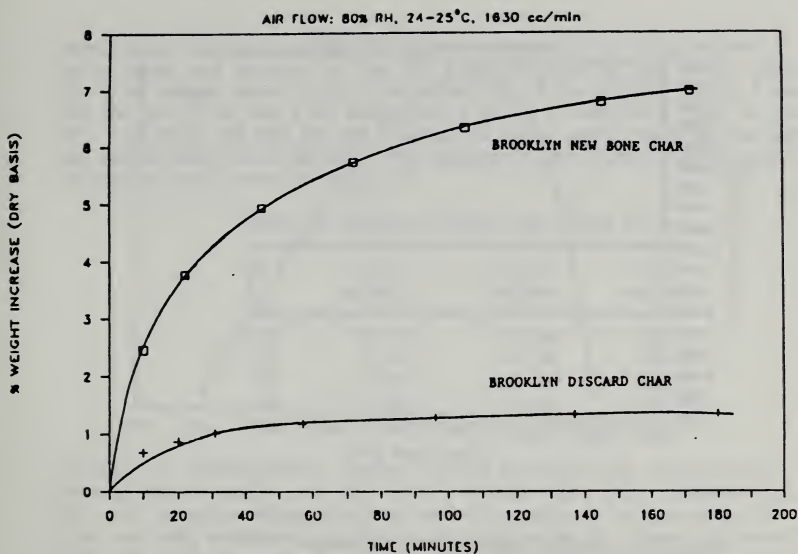


Figure 1.--Water take-up of new and discard bone char.

#### Rate of Water Take-Up

The above measurements at constant relative humidity and temperature are concerned with the fraction of the total water take-up by the oven-dried sample of the carbon (g per 100g of dry sample). The Langmuir kinetic model was assumed and expressed as follows:

$$\frac{d\theta}{dt} = k_1 (1-\theta) - k_2 \theta$$

where  $\theta$  is the fraction of the total weight increase ( $w_\infty$ ) at a specified time  $t$ . When integrated within the limits of  $\theta = 0$  for  $t = 0$  and  $\theta = w/w_\infty$  for the steady state at  $t_\infty$ , the following relationship holds:

$$\ln \frac{w_\infty}{w_\infty - w} = kt, \text{ where } k = k_1 + k_2$$

A plot of  $\ln [w_\infty / (w_\infty - w)]$  versus  $t$  was linearized by a computer calculation using different values for  $w_\infty$  until the calculated statistical coefficient was maximum, usually 0.999. An example is shown in Figure 2 for the water take-up by the sample of Brooklyn discard char. The resulting linear plots for the two samples mentioned in Figure 1 are given in Figure 3.

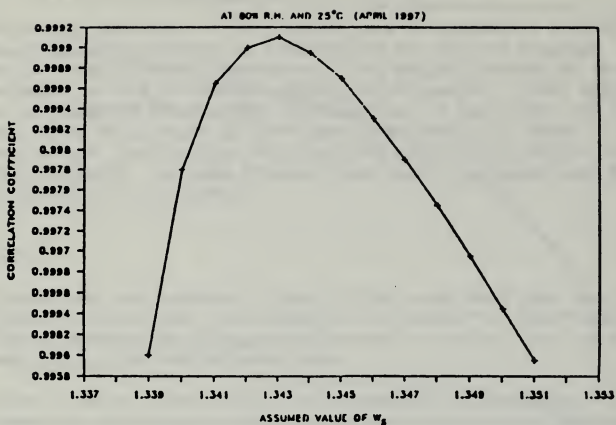


Figure 2.--Water take-up by a Brooklyn discard char.

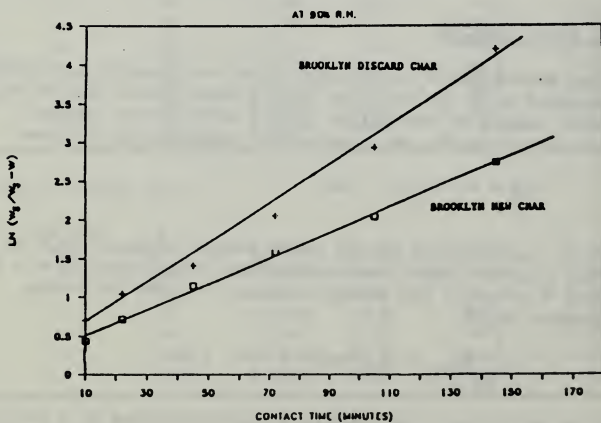


Figure 3.--Comparison of water vapor pick-up of new and discard char.



Under the conditions of the present measurements a large excess of water vapor was present in the air flow relative to the water take-up. The sample space (1.9 cm diameter and 3 cm height) was 8.5 cm<sup>3</sup> and the air flow was 1.63 L/min which gives a residence time of 0.31 sec. At 25°C and 80% RH the content of water vapor passing through the sample was 18.45 mg/min. The amounts under other experimental conditions are given in Table 2.

Table 2.--Mass of vapor per cubic meter

RH %	20°C mg/min	25°C mg/min	30°C mg/min
100	17.30	23.06	30.39
90	15.57	20.75	27.35
80	13.84	18.45	24.31
50	8.65	11.53	15.20

To date, 17 measurements (Table 3) have been made on water vapor take-up for 8 samples of char with a known batch color removal (%). The rate of water vapor take-up can be calculated at any specific time and that at 20 minutes has been correlated by linear regression with the color removal results. Also, the calculated steady state water take-up ( $W_s$ ) has been correlated with the corresponding color removal (Table 3). The slope (m) and intercept (i) of the regression line are given at the bottom of Table 3.

The standard deviation of four determinations of water vapor take-up with the #4 Baltimore char is given in Table 4.

Table 3.--Correlation of water take-up with the batch test color removal.

Char	Color removal	Water Pickup	
		Rate (20 min) mg/g	Steady state mg
New Char 1	98.0	0.052	8.0
New Char 2	97.4	0.048	7.30
#1 Baltimore	71.1	0.039	5.50
Brooklyn	64.8	0.023	3.15
#4 Baltimore	61.4	0.014	2.26
#3 Baltimore	52.1	0.027	2.77
Discard Baltimore	20.8	0.0116	1.34
Discard Baltimore	18.8	0.0087	1.68
Regression line parameters		n = 8 $r^2$ = .907 m = .000507 i = .0028	n = 8 $r^2$ = .912 m = .0788 i = .770

Table 4.--Reproducibility of water pick-up measurements (#4 Baltimore char).

	Rate (20 min) mg/min	Ws mg
	0.0134	2.320
	0.0137	2.325
	0.0152	2.285
	0.0152	2.128
Mean	0.0144	2.264
Standard Deviation	0.00083	0.080

#### DISCUSSION

The correlation coefficient ( $r^2$ ) of the rate of water take-up at 20 minutes with Batch-Test Color Removal (Figure 4 and Table 3) is 0.907. The pairs of measurements were made in independent laboratories and by separate operators with sub-samples of the chars. The one outstanding deviation is with the #4 Baltimore sample. For this sample, the activity of carbon is probably the dominating factor in decolorization. If this sample were omitted (no apparent justification), the correlation coefficient would be  $r^2 = 0.967$ .

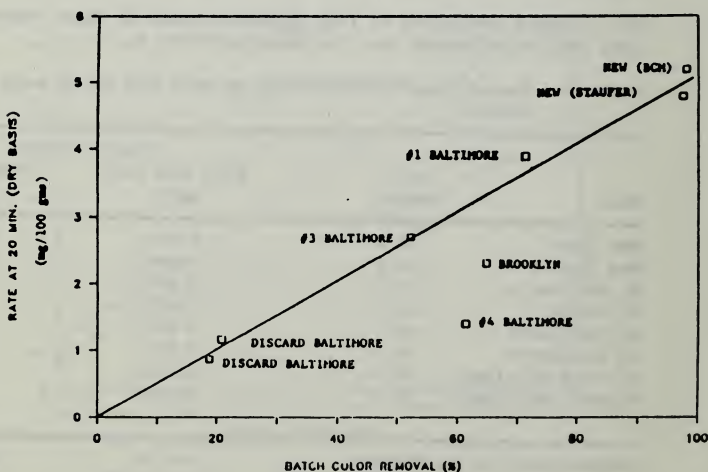


Figure 4.--Correlation of rate of water take-up (20 min) with batch color removal %.

Chou (1983), in an earlier study, suggested that the mechanism by which sugar colorants are adsorbed onto a carbon surface may be divided into four consecutive steps as illustrated in Figure 5, namely dispersion, interparticle film diffusion, intra-particle diffusion, and adsorption onto surface sites. The rate of decolorization depends on the relative significance of these sequential steps. The slowest step becomes the rate determining step in the overall rate process. For a diffusion control step, the variables affecting the diffusion may be correlated by an expression derived from the Stokes-Einstein equation as follows:

$$D = \frac{F(T)}{\mu V^n}$$

where  $D$ , the diffusion coefficient, is a function of temperature,  $T$ , viscosity,  $\mu$ , and the molecular size of the colorants.

The observations from the present study indicated that an additional diffusion step, i.e. diffusion across the adsorbed water layer on the carbon surface may be suggested.

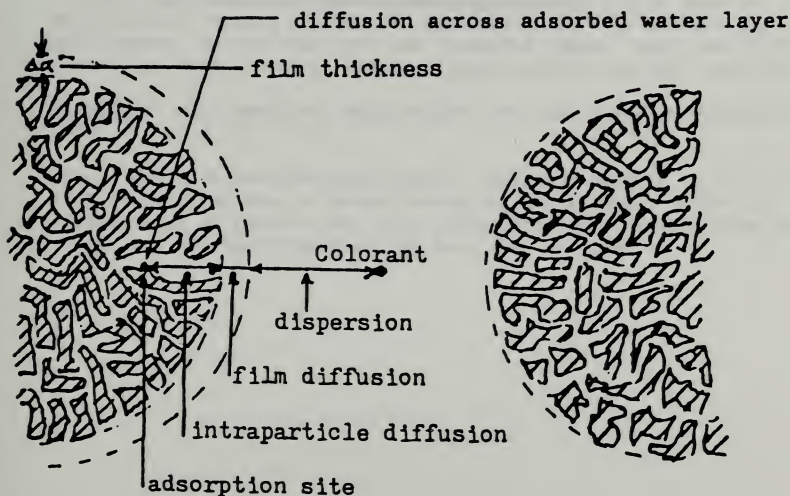


Figure 5.--Adsorption model for a particle of bone char.

The observed correlation also raises several questions as to its significance and importance. Is bone char completely wetted by 65 Brix sugar liquor? Does bone char have a porous system available to water vapor and only partially so to sucrose and color bodies?

The dependence of the water vapor take-up by adsorbent carbons on time, relative humidity and temperature is well known, but the influence on composition of the carbon surface on bone char is less well understood. The carbon matrix of bone char contains combined hydrogen, oxygen, nitrogen and sulfur. The basic calcium phosphate matrix can furnish a highly polar surface and hence influence the water vapor adsorption. What occurs during the many cycles of regeneration adds further complications, but the main observation is that it modifies the take-up of water vapor.

The desirable objective of kiln treatment in regeneration is to end up with a boundary surface that is highly polar and with no loss of surface area and color-removal efficiency. It appears that the rate of water vapor take-up is a measure of the approach to such a goal.

#### REFERENCE

Chou, C.C.

1983. Criteria for bone char evaluation. Proc. 1982 Sugar Processing Research Conf., pp. 24-46.

## DISCUSSION

Question: In the microporous system, is the water pick-up a pure condensation process?

Chou: It should be a combination of adsorption and condensation. For mono-molecular layer water pick-up, adsorption would dominate the system.

G. Irvine, British Charcoals & Macdonalds: Thank you for an excellent paper on bone charcoal. We have looked at the relationship between the batch test for color removal and the surface area and also the density of a char, and I have some figures to demonstrate this.

(Fig. 1): On the bottom axis, we have the color removal by the batch test, and on the vertical axis, we have the readily accessible surface area. These curves form the same pattern as you got with your water absorption test against the color removal.

(Fig. 2): On this figure we look at the density of bone char against color removal. Again, it can be seen that there is almost the same pattern.

I think you were wise to discard that heavy material, as it contributed very little to the over-all activity of your stock char. I would urge anyone using bone char not to be afraid to take out heavy material and replace it with new bone char. It will enhance your performance, and it will save you financial chaos. Don't be afraid to throw char away--there is plenty of good quality new char available.

Question: What practical application does your water absorption model have?

Chou: Based on the adsorption model, diffusion appears to be the rate-determining step at normal operating conditions. Therefore, optimization of temperature, Brix, flow rate and retention time should be the key for improvement of the bone char process.



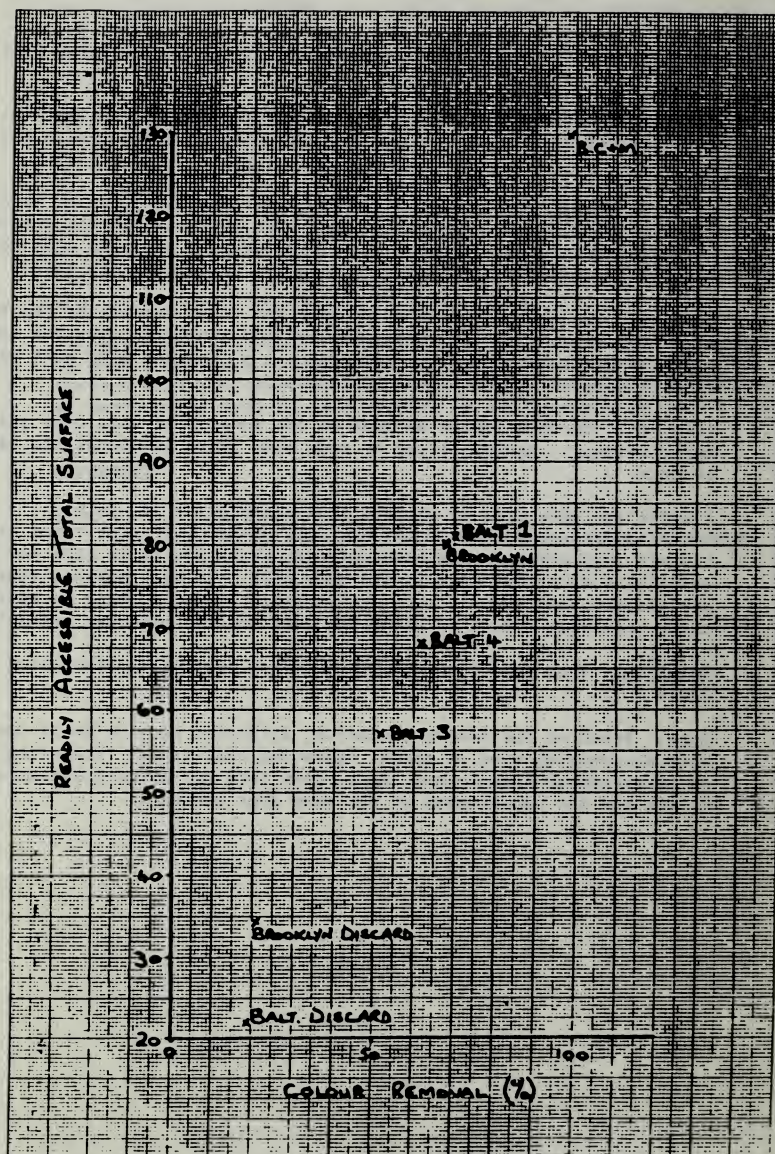


Figure 1.--Color removal versus readily accessible surface area.



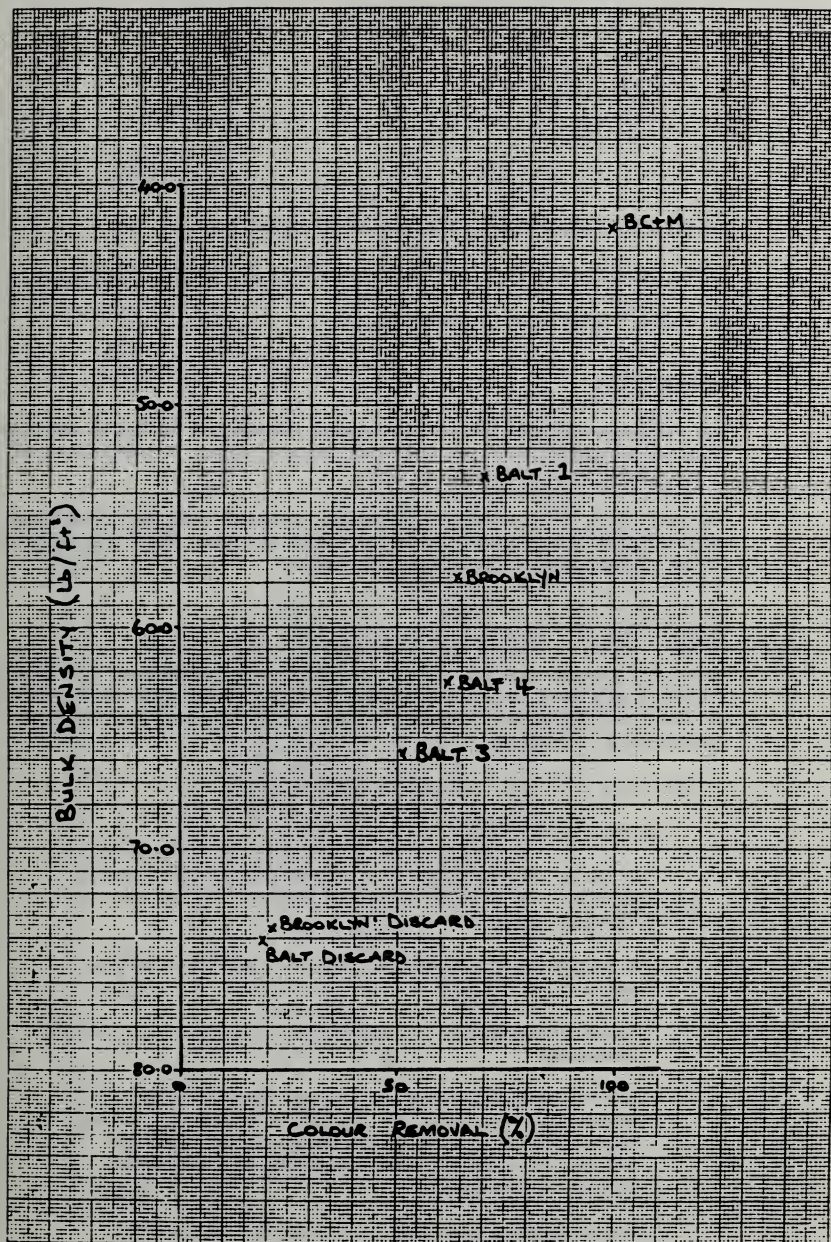


Figure 2.--Color removal versus char bulk density.

## CONTINUOUS VACUUM PAN OPERATION AT COLONIAL SUGARS, INC.

E. O. Betancourt, J. L. de Chazal, and J. D. McCulla

Colonial Sugars, Inc., Gramercy, LA

### INTRODUCTION

The operation of the first continuous vacuum pan in North America started in August 1987 at Colonial Sugars, Inc., a subsidiary of Savannah Foods & Industries, Inc.

The ease and flexibility of operation coupled with the increased remelt sugar quality and yield obtained has made this pioneering effort a worthwhile step into the future.

### DESCRIPTION OF EQUIPMENT

Presently, there are four types of Langrensey Continuous Vacuum Pans (LCVP). All have evolved from the Type L (see Figure 1) which will be described in order to make it easy to understand the Type CA installed at Colonial.

LCVP Type L -- The equipment consists of:

- . Two vertical walls "A".
- . A bottom and a dome "B". These are rounded in the case of the straight pan and conical in the case of circular pans.
- . A calandria "C" mounted against one of the vertical walls.
- . An inclined plate "E" above the tube sheet to contain splashing.
- . A feedstock distribution system consisting of a horizontal manifold "F" feeding a series of dosing tubes "G" to introduce the feed syrups below the calandria at calculated distances throughout the length of the vessel.
- . All necessary pipes and fittings for magma and steam inlets, massecuite, vapor and condensate outlets.

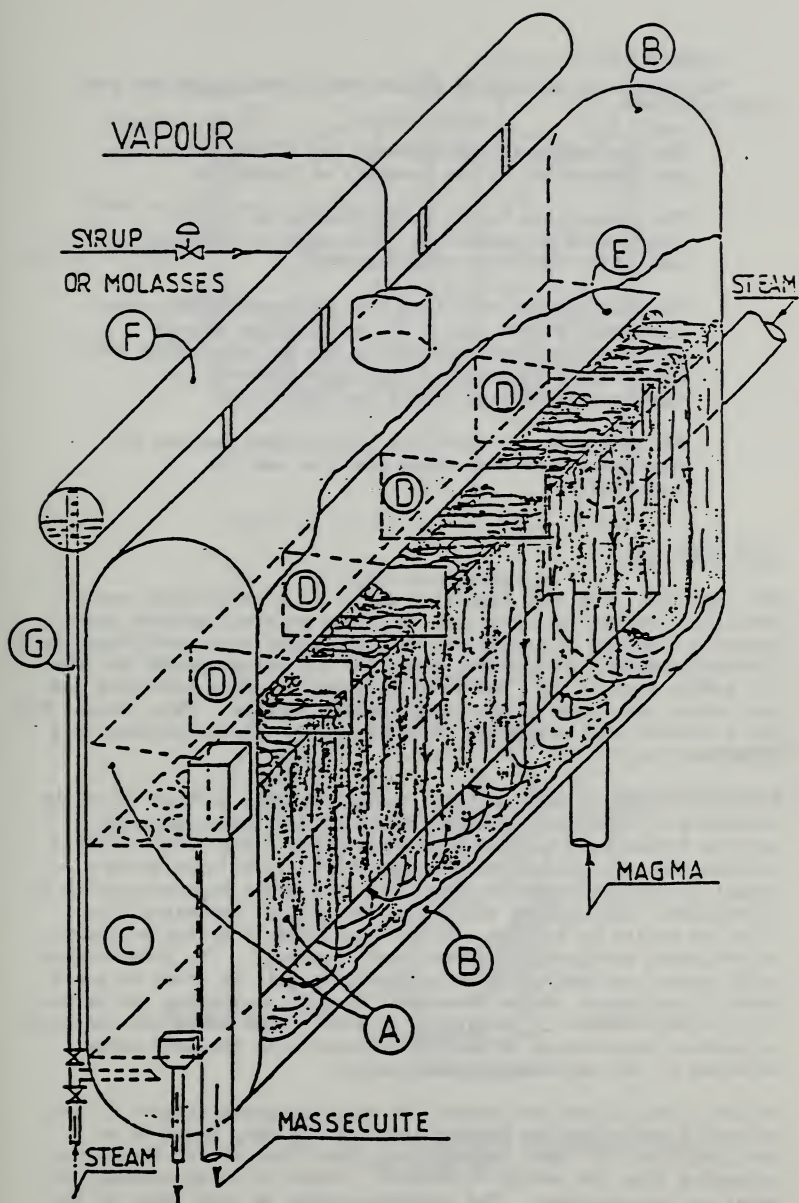


Figure 1.--Diagram of Langreny Continuous Vacuum Pan Type L.



The operating principles of the continuous vacuum pan are simple:

- . The seed magma is continuously fed at one end of the pan, controlled by the quantity of feedstock.
- . The evaporation in the tubes results in lateral circulation of massecuite (from tube to downtake) similar to the batch calandria pans.
- . The displacement resulting from the seed magma volume introduced at one end of the pan and the crystal growth taking place throughout the pan results in a longitudinal movement of massecuite towards the opposite (discharge) end of the pan.
- . The independent lateral and longitudinal motions of massecuite described above give the pan its versatility.

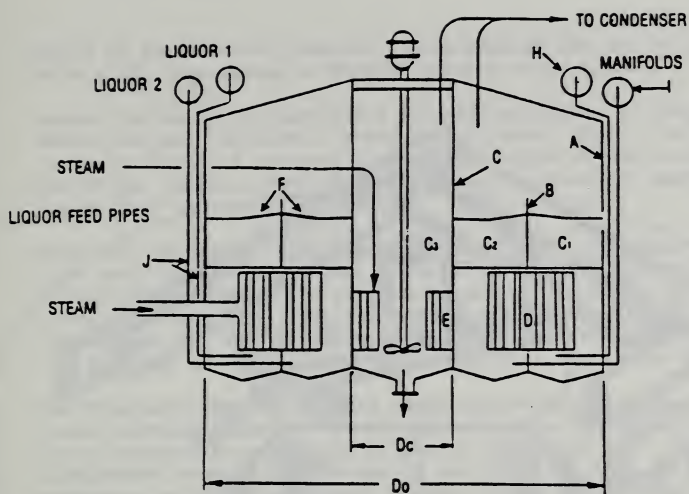
#### LCVP TYPE "CA"

The type "CA" LCVP (see Figures 2 and 3) is a cylindrical vessel with three massecuite circuits. A larger external annular space houses a floating calandria "D" with its sugar side partitioned by a circular vertical baffle that separates the outer circuit C1 from the middle circuit C2. The inner cylinder houses a calandria pan with about one-sixth of the total heating surface. This circuit C-3 has a stirrer and is capable of "heavying up" the massecuite to a high Brix.

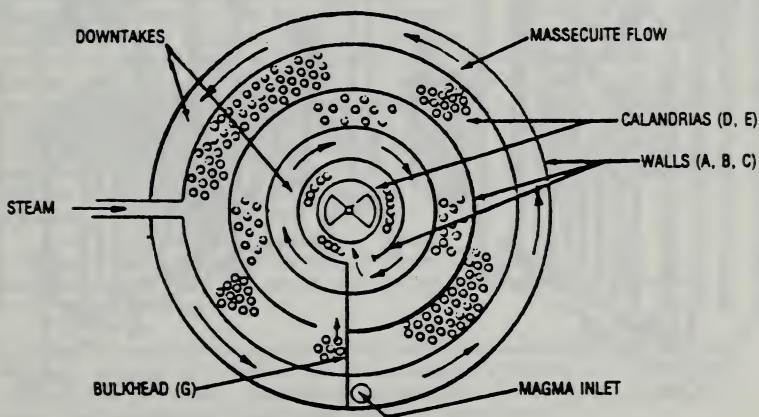
Circuits C1 and C2 have vertical cross-sectional baffles above the calandria to contain sideways movement. These cross-sectional baffles divide each circuit into six sectors. Every sector has a sampler and a Resistance Temperature Device (RTD) that sends a signal to the programmable controller from which supersaturation is calculated. The heating surface of the sectors increases from inlet to outlet to achieve a higher boiling rate of the massecuite as the grain develops and moves toward the exit. The tubes within each sector are laid radially and equidistantly in order to avoid dead or cold spots in the calandria, thereby maximizing the natural circulation. Sector 1 is baffled in five places above the calandria to prevent intermixing of growing crystals with the seed magma entering at the beginning of this sector.

On the circular vertical baffle "B" that separates Circuit 1 from Circuit 2 and above the working level of the pan, there is an inclined plate "F" that prevents carryover from either circuit or splashing from one sector to another. There is no entrainment of sugar to the condenser. The calandria tubes on both sides of the circular vertical baffle "B" are close to the downtakes, which enhances massecuite circulation throughout the pan. Below the calandria, baffle "B" totally supports the weight of the calandria





Vertical Cross Sectional View



Pan Cross Sectional View

Figure 2.--Diagrams of Langreny Continuous Vacuum Pan Type CA.

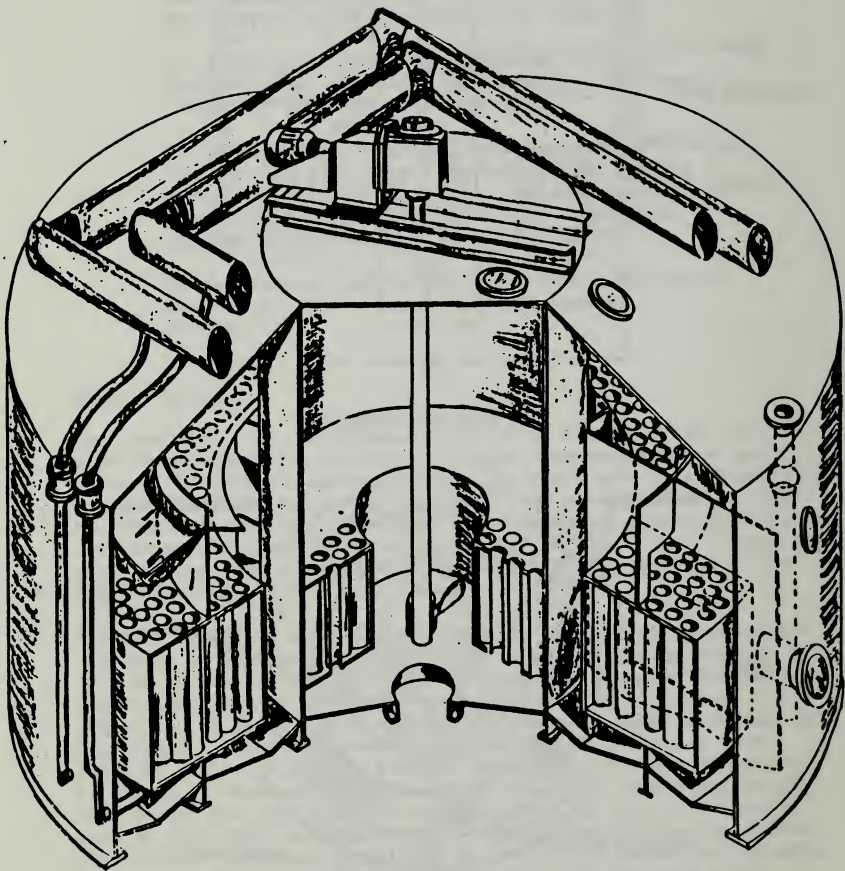


Figure 3.--Three-dimensional drawing of the pan.

while it separates C1 from C2. The bottom of the pan is conical with a "W" type bottom to further enhance circulation.

Liquor is fed to the massecuite from two manifolds "H" located above the pan. Manifold 1 feeds higher purity liquor 1 to Circuit 1, while Circuit 2 receives lower purity liquor 2 from manifold 2. Facilities exist to feed liquor 1 at the beginning of manifold 2 or liquor 2 at the end of manifold 1.

There are 30 dosing tubes inside each manifold. Five dosing tubes feed each sector. The dosing tubes of each sector have orifices calculated to provide the adequate amount of liquor for the heating surface of the sector. The dosing tubes introduce feed liquor below the calandria. The incondensable gases of the calandria vent into the manifold and warm up feed liquor on their way to the condenser.

The continuous feeding of seed magma and the crystal growth taking place throughout the pan are translated into a longitudinal movement of the massecuite toward the discharge end of the pan. The independent lateral circulation up the tubes and down the annular downtakes and the longitudinal movement along the massecuite path give the pan its versatility.

When massecuite arrives at the end of Circuit 1 in Sector 6, it travels through openings on the vertical baffle both above and below the calandria. It is now in Circuit 2, Sector 7, and travels toward Sector 12 located opposite to Sector 1. (Liquor from Manifold 2 is fed just as in Circuit 1.) When the massecuite arrives at the end of Sector 12, it overflows through a "U" tube that has a high or low level adjustment into C3. Since boiling in C1 and C2 is done at higher absolute pressure than C3 and since there is a difference in elevation in the C3 calandria pan, massecuite travels to C3. This "U" tube with the level adjustment creates a hydraulic seal that permits C3 to work with less massecuite level than C1 and C2.

A connection is provided at the "U" tube that permits the introduction of run-off syrup that helps lubricate the massecuite as it moves through.

Upon the massecuite entering C3 and with the mechanical circulator running, mixing of crystals is prevented by a baffle at the outlet side of the "U" tube. Splashing is prevented by a plate mounted above the massecuite level over the "U" tube only.

In C3 final tightening of the massecuite is achieved; it overflows through a high-low level adjustable weir and enters a barometric column that discharges into a volumetric pump. The level in the barometric column is maintained by a level controller that also controls the discharge pump's speed.

The pump is a volumetric, non-modulated, gear pump, self-priming with lobe rotors turning in a contrary direction, one with regard to the other, and thereby drawing material by the periphery. Since the lobes counter rotate, sugar crystal breakage is minimal.

#### INSTRUMENT CONTROL

The continuous vacuum pan is controlled from an operator's station by a Foxboro Microspec 200 Microprocessor with eight multi-display stations and three 4-pen recorders. This programmable controller is self-timing and has remote and local set points. The steam flow to the C1-C2 calandria is measured by a flow meter. The liquor input to the pan is measured by three magnetic flow meters. The seed magma input is determined by calculation from the revolutions of the volumetric pump.

The absolute pressure in C-3 is controlled by modulating the flow of injection water to the condenser; the absolute pressure in C1 and C2 is controlled by modulating the vapor flow control valve to the condenser. High and low pressure values energize audio-visual alarms at the annunciator panel.

The master control loop is the steam flow to the C1-C2 calandria. A base amount of magma to Sector 1 of C1 and liquor to C1 and C2 manifolds are independently ratioed to the steam flow. Thus, as the pan is sped up (by increasing the steam flow setting) or slowed down, the equilibrium is maintained by the mentioned ratios.

Supersaturation calculations are available for each of the 13 sectors of the pan and C3 inlet. A six-point switch for C1 and another for C2 allows the operator to check the temperature and supersaturation of each sector. Supersaturation in C1 and C2 is controlled by controllers which modulate an amount of liquor above the basic input ratioed by the steam. This completes the total amount of liquor pumped to C1 and C2 manifolds.

An RTD at the "U" tube outlet measures the temperature of massecuite as it emerges in C3. This signal is used to compute supersaturation at C3's absolute pressure and is fed to a controller which modulates a feed valve to the "U" tube in order to control C3 inlet supersaturation at the desired level.

The final Brix of the massecuite leaving C3 (Sector 13) can be controlled by the supersaturation of the sector or switched to the mobility reading derived from the agitator motor load. A feed valve discharging under the agitator is modulated by this controller.

In order to compensate for purity variations in the feed materials and enable operators to target desired product purity, a selected flow rate of liquor 2 can be fed at the end of liquor 1 manifold or liquor 1 can be fed at the beginning of C2 manifold. A controller which takes the signal from a magnetic flow meter regulates the flow to maintain the flow rate at the set point.



Three 4-pen recorders keep track of the operation: #1 records supersaturation in C1, C2, C3 and C3 inlet; #2 records flow rates of liquor 1 to C1, liquor 2 to C2 and liquor 2 or 1 to C1 or C2, and steam flow rate; #3 records absolute pressure in C1, C2, and C3, mobility in C3, and condensate flow out of C3 calandria.

## OPERATION

The continuous vacuum pan has enhanced Colonial's remelt operation. Since it can be set between 50 and 150% of its design capacity, it gives the operation people a very useful edge. It responds well to changes of pace although it is advisable to maintain the rate of operation as consistent as possible by careful planning.

Seed grain is boiled in a batch pan and stored in a seed mixer. A variable speed volumetric magma pump provides the continuous vacuum pan with an uninterrupted stream of seed grain at the preselected flow rate.

Colonial uses the continuous vacuum pan for high remelt boiling. C1 is fed with liquor 1, a mixture of concentrated sweetwater and affination syrup with a normal purity of 83 that can fluctuate between 80 and 86. C2 is fed with liquor 2 which is the separated wash portion of the high remelt centrifugals and repurged crystallizer sugar. The usual purity of liquor 2 is 62 but it can vary between 59 and 65. Finally, C3 boils back the syrup from the remelt strike normally at 51 purity which can vary 3° above or below.

Prior to the continuous vacuum pan, Colonial used a two boiling system with high remelts of about 72 purity and low remelts of about 62 purity. About 15% of the high remelts were high high's of 83 purity approximately. These "hi-hi's" were needed to bring down high purities generated on shutdown or by very high pol raws. Since the advent of the continuous vacuum pan, the high-highs have been eliminated.

The continuous vacuum pan offers a great deal of flexibility to control the purity of the massecuite or to cope with process or raw sugar abnormalities. It has the capability of being set to feed liquor 2 at the end of liquor 1 manifold or feed liquor 1 at the beginning of liquor 2 manifold. C3 normally boils back high remelt syrup of 51 purity but can be changed to high remelt wash to increase the massecuite purity or to avoid high viscosities. If necessary both manifolds can be fed with liquor 1 or 2.

The continuous vacuum pan is capable of boiling a tight or heavy massecuite. The closeness of all its tubes to the downtake and normal low massecuite level allows the pan to boil heavy without a great loss of circulation. C1, with its higher purity, can boil at



93 Brix, C2 at 93-94, and C3 at 95 to 96.5. C3 is capable of producing 98 Brix massecuite if desired. These Brixes are determined with a hydrometer on a double dilution.

The pan can be operated for several weeks without stopping. On weekend shutdowns C3 can be liquidated and C1 and C2 stopped full and restarted without difficulty two or three days later.

It is easy to attain the desired grain size by adjusting the ratio of seed to steam. The piston-like movement of massecuite within the pan allows good seed magma to grow into excellent sugar. Mean apertures of 0.77 mm in the high remelt grain have been easily attained. Normally, the target size is 0.40 mm mean aperture. The C.V. of the massecuite fluctuates between 26 and 36 depending on seed grain uniformity.

## DISCUSSION OF RESULTS

Observing the temperature graphs for the averages of the fourth quarter of 1987 and first and second quarters of 1988 (Figure 4), a slow increase can be seen from Sectors 1 to 12 as sucrose is crystallized and the purity of the mother liquor decreases. We feel that RTD's in Sectors 4 and 8 are slightly in error. Due to the lower absolute pressure in C3 (Sector 13), its temperature is lower.

Prior to the start of the continuous pan, the high remelt sugar purity averaged 96.56. The quarterly averages of the continuous pan high remelt sugar purity are:

Fourth Quarter 1987	--	96.64
First Quarter 1988	--	96.93
Second Quarter 1988	--	96.81

This noticeable improvement has been attained without increasing the wash water of the continuous centrifugals.

The bar chart in Figure 5 shows the average purity drop between massecuite and syrup for the high remelt strikes after 8 to 12 hours in air cooled crystallizers. The average purity drop for the 4,454,000 cubic feet boiled in 3.5 years was 19.73%, while the 620,000 cubic feet boiled with the continuous pan in three quarters was 22.27%. This means an increase of the exhaustion of 2.54 purity points in the high remelt station, a 12.9% improvement.

# CONTINUOUS VACUUM PAN

TEMPERATURE BY SECTORS

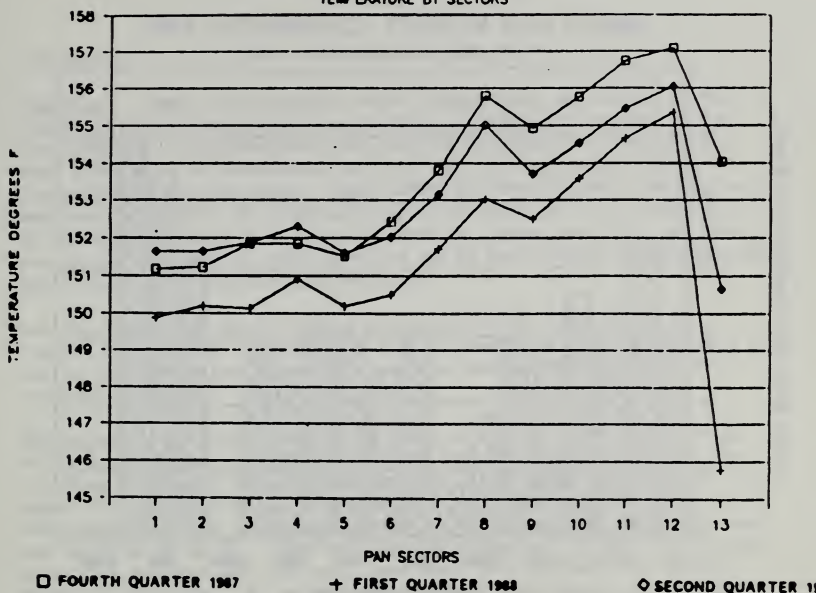


Figure 4.--Temperature in pan sectors for three quarters.

## CONCLUSIONS

The steady run of the continuous pan has contributed to balance the peaks and lows in exhaust steam pressure. One of the three live steam makeup valves has been closed and nobody has missed it.

The exhaustion in the high remelt station has improved by 13% while the polarization of the sugar has increased.

Most of all, this installation has put the operating personnel in complete control of quantity and quality of the remelt boiled at the pan floor.

At Colonial we feel our continuous vacuum pan project has set a new standard for boiling sugar in North America.

# BATCH PAN VERSUS CONTINUOUS PAN

PURITY DROP BY QUARTERS (1984-1988)

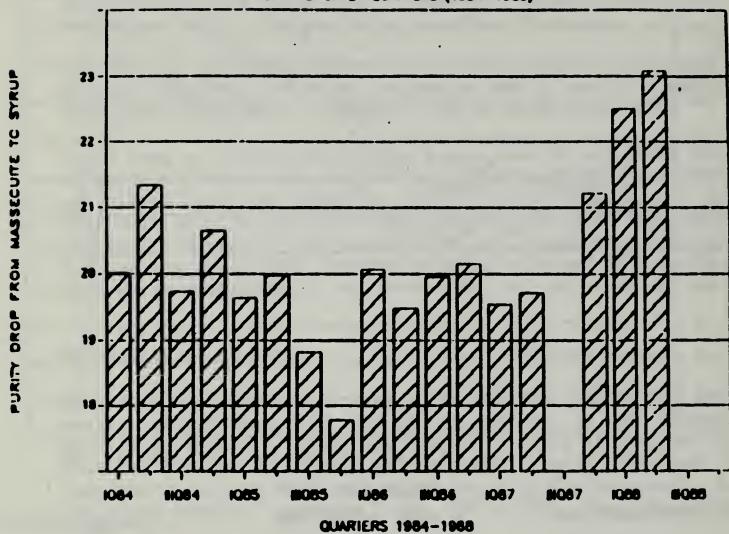


Figure 5.--Average purity drop between massecuites and syrup for the high remelt strikes after 8 to 12 hours in air cooled crystallizers.

## DISCUSSION

Question: There are probably three things that have delayed the introduction of continuous pan boiling. Those are crystal size distribution, encrustations and control problems. I understand that your flow of massecuite is such that your crystal size distribution is very good. What about encrustations? You did not mention the purity of your massecuite--I imagine it is around 75. Do you have trouble with encrustations at these purities, or have you boiled the pan at high purities or do you intend to because that would be a very interesting exercise.

Are you satisfied with boiling point elevation as a means of control? Were you feeding in a bit of seed into the pan? Would it not be better to try to measure crystal content rather than the supersaturation?

Betancourt: We boil at about 72 purity, but if, for some reason, we have an excess of No. 1 liquor, we can go to 74-76 purity, and our drop in purity does not suffer much from those variations. It seems that the pan boils so vigorously that the encrustations are very small. About every 3 weeks or so, we liquidate the whole pan and boil water in it. The two outside circuits, C1 and C2, in the year that we have had the pan, have been boiled once with a mild Mecalene solution. We have boiled C3 with mild Mecalene solution three times. This pan is a "race horse", and we don't have enough liquor for it.

There has been no problem with caking of sugar, although the continuous vac. pan has operated up to three weeks non-stop. Once, with a very high Pol raw which yielded little affination syrup, on the third week of slow pan operation, some small lumps were noted in the massecuite.

During a four week trial, 98 purity white sugar massecuite was boiled in this pan. At the end of each week, some caking had taken place above the massecuite level. Changes made for the white sugar model have eliminated this problem. There is a white sugar pan now operating in Belgium.

Regarding the control, I like the mobility in C3. Supersaturation seems to be enough for remelt, although at times when there are serious variations of purity, we have to go back and forth and check it against actual samples.

Question: How do you control the level in C3, and how do you control the final RDA or density?

Betancourt: We control on two levels--a high level and a low level. We lower a wier when we want a high level. The wheels that I showed in the slides at the beginning of the talk are the control with which we raise and lower the level. The low level is about 18 inches above the calandria, and the high level is about 30 inches above the calandria. The final brix is controlled by mobility derived from agitator motor load.



## OPERATION AND CONTROL OF COMBINATION CHAR AND CARBON SYSTEMS BY ONE PERSON

Richard Priestler

Savannah Sugar Refinery

Oliver Lyle in his outstanding book, *Technology for Sugar Refinery Workers* (1957) tells of a Tate and Lyle director who was one day watching the construction of a new bridge. He was especially interested in observing three riveters working with a pneumatic hammer. An unemployed man came by and said, "If it wasn't for that bloody thing, there would be ten men working there instead of three." To which the director replied, "If it wasn't for that bloody thing, there would be no bridge built, and those three men might be looking for jobs."

People are naturally apprehensive when they begin to see cut-backs in positions. In the long run, however, they and everyone else will benefit. The primary reason for trying to accomplish such labor savings is not to expel people from gainful employment, but to increase productivity per individual worker.

In this regard, Mr. Lyle wisely asserts, "Labor saving does not necessarily mean labor dismissals. ... Every man saved makes the jobs of those remaining more secure, and there is a good chance that the 'saved' man will either not be dismissed or will soon be re-engaged for some other enterprise." Mr. Lyle then supports his claims by giving some statistics from one of their operations. He cites from a 25 year period where over 650 jobs were eliminated. However, the actual number employed grew substantially, and the output per person rose by over 60 per cent.

Savannah has subscribed to this philosophy for many years. We have added equipment and automated entire stations. This has resulted in reduced work loads for both foremen and operators which have enabled us to combine numerous responsibilities. From this has evolved not only an increased contribution from each individual, but in addition, a considerable rise in production from the entire refinery. During this time there have been no lay-offs, and the working environment for plant personnel has improved significantly. Because of such dramatic achievements, Savannah's employees were presented in 1985 with the United States Senate's Productivity Award.

The purpose of this paper is to share one of Savannah's most recent accomplishments in productivity gains. Through reorganization and equipment modifications, the entire "heart of the sugar refinery," which consists of the Char and Carbon Houses, is now able to be operated and controlled by only one person.

To better comprehend how this was done, it is first necessary to have a basic understanding of this process phase. Figure 1 shows a flow diagram of the combination char and carbon systems at Savannah Refinery.

A solution of washed raw sugar which has been carbonated and filtered to produce an amber colored crystal-clear liquor at 65.0 Brix serves as the input to the Char House. Located here are 42 filters, each having a capacity of 32.5 tons of char and 5,000 gallons of sugar liquor.

The inlet liquor flows through 30 of these filters in parallel, each receiving 22 to 25 gallons per minute. This assures the needed process flow of 700 gallons per minute. Liquor is passed through each filter for a period of 100 hours. By this time the char's adsorption ability has now become ineffective due to the accumulation of impurities. Thus, effluent from this material can no longer be allowed to go forward.

The other 12 filters are at various stages of (1) "sweetening off" to remove virtually all of the sugar present from the expended char, (2) washing to expel as much of the collected impurities as practical from this material, (3) "blowing down" to dispense of most of the remaining water, (4) emptying of the spent char so that it can be sent to the kiln for revivification, and (5) filling with reactivated material from the kiln.

After a filter is "sweetened off" and washed, the char is conveyed to a seven hearth Nichols-Hershoff Kiln. At the seventh hearth, char temperatures have climbed to 1000°F with an oxygen level of 2-3%. A density table located between the kiln and filters removes exhausted particles from the revivified material. Replacement with new char amounts to approximately 6,000 pounds per day.

From the Liquor Gallery, effluents from the 10 newest filters move ahead in the main process flow. These filters contain char which has been exposed to sugar liquor from 0-33 hours. The remaining 20 filters have been "on liquor" from 33-100 hours. Their exit streams are sent to the carbon system for additional decolorization.

The Carbon House consists of seven 1500 cubic feet stainless steel filters. A flow of 80 gallons per minute per filter is maintained through 6 of these vessels operating in parallel. Target color removal is 50-60%. Under these conditions, "on liquor" time for each filter is 400 hours. Exit streams from these filters are

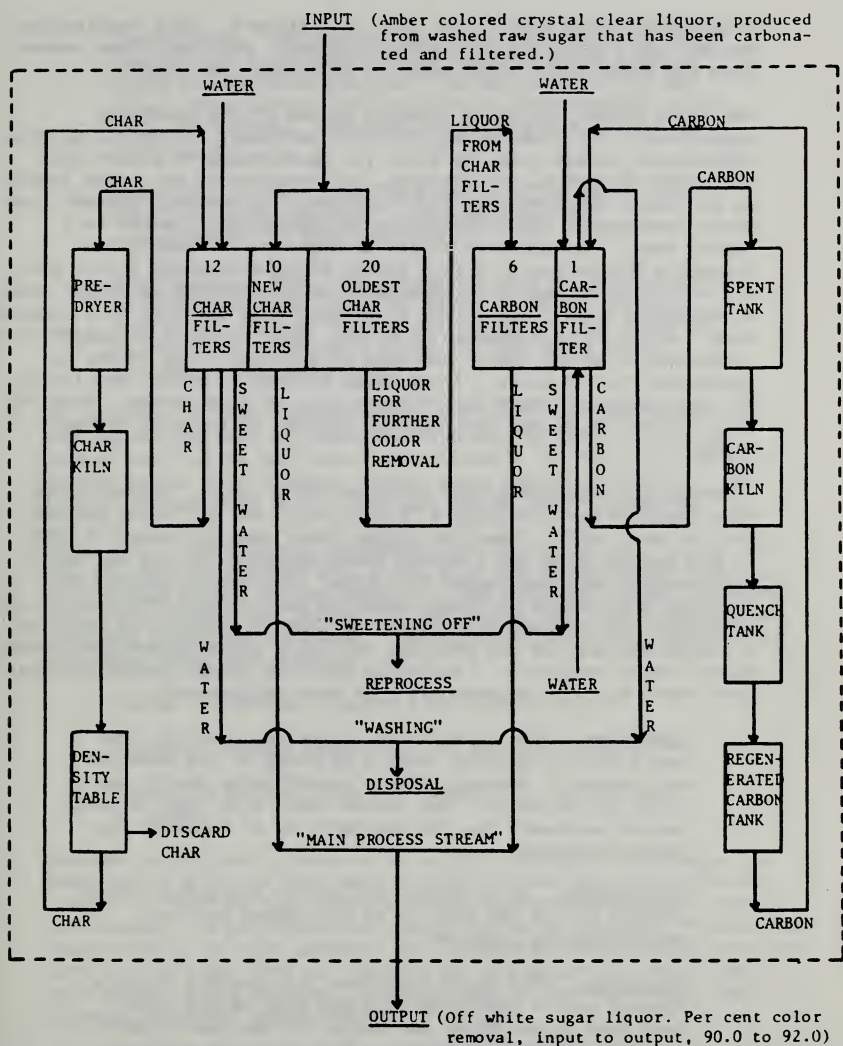


Figure 1.--Flow diagram of combination char and carbon systems at Savannah Sugar Refinery.

merged with those from the 10 best char filters. This combination becomes the refinery's main process stream. Per cent color removal from the input to the Char House to this point is 90-92%.

The other carbon filter is involved in one of the following activities: (1) "sweetening off," (2) emptying, (3) refilling with reactivated carbon from the kiln, (4) backwashing to remove fine particles of carbon, (5) draining, (6) "sweetening on," with liquor in and sweet water out, or (7) "on liquor" to the main process flow. After "sweetening off," the carbon is hydraulically moved to a "spent tank." An inclined scroll is employed to dewater and transport the carbon to a five hearth Nichols-Hershoff Kiln. Here the carbon is raised to a temperature of 1750°F with an oxygen level of virtually zero. The carbon is dropped from the kiln to a "quench tank," where it is submerged in water. From there it is pumped to a "regenerated carbon tank," and then to a filter. The carbon is backwashed for 12 hours. After being drained of water, the filter is "sweetened on," with the effluent sent to sweet water. When the Brix reaches 50.0, the discharge is changed to the main process stream.

Savannah has employed a Char House for many years. Operators for this function have included a "filter top" and a "filter bottom" man for each shift. When the Carbon House went on stream in 1985, it was necessary to add a Carbon House Operator for the day shift. To determine what was needed for better operational control and improved utilization of labor, Savannah made a detailed study of its equipment and job responsibilities on these stations. In addition, a review was made of the problems that were being encountered during normal daily activities. Following are some of the conditions that were found and the improvements that were implemented:

1. Condition. When a filter was being emptied, the char would flow by gravity until a majority of the material was removed. However, when a considerable amount was still in the vessel, the stream would stop, and it would become necessary for the remainder to be manually dug from the filter.

Improvement. An automatic digger was installed. This enabled all of the char to be discharged with no manual digging required.

2. Condition. Char would flow from the predryer through louvers onto a vibrating tray and then to the kiln. Feed rate to the kiln, and hence, to the char filters could be extremely variable. Char from the predryer would, on many occasions, be either too wet or too dry. When too wet, it would clog the louvers, and stop the flow. When too dry, it would overrun the louvers.

Also, fires would develop in the predryer, which had to be extinguished. All of this required close monitoring and attention.



Improvement. The louvers and the vibrating trays were removed and replaced by three variable speed scrolls, which now dispense the char from the predryer to the kiln. This assures more consistent feed control to the kiln and filters. Predryer fires have also been eliminated.

3. Condition. Discard char was put into 100 pound bags. Each had to be sewn shut. This amounted to approximately 20 bags per shift.

Improvement. This time consuming task was discontinued by discharging the material directly into a box of sufficient size to contain all of the discard produced on one shift. Disposal units are now large boxes instead of 100 pound bags.

In addition to these improvements, the char kiln, the carbon kiln and the boiler were all placed on a Bailey Network 90 control system that can be monitored with virtually no additional efforts by the Boiler Room Foreman. Thus, these responsibilities do not have to be attended by the Char and Carbon House Operators.

After this revamping was completed, the duties of the "filter top" and the "filter bottom" man, along with the Carbon House Operator were merged into a single position. Now only one person is needed for the operation and control of the entire Char and Carbon House, plus the firing of the kiln. From this the following benefits are currently being experienced:

1. Better operational controls for the stations;
2. Improved working conditions for the operators;
3. Increased productivity per person for this process phase.

Extreme competitive pressures in recent years have challenged our industry to a degree that many refineries have had to permanently close. So that we can remain a viable contestant in such a market environment, it has become necessary for us to carefully examine our operations to see how expenses can be reduced to the lowest possible levels. This has been the impetus for the activities described in this paper, since the most effective way to lower per unit costs is through increased productivity.

Much has been said about the triumphs of Japanese industry, and how their accomplishments in this regard have driven many competitors in this country out of business. In response, and out of a spirit of pessimism have come such remarks as, "We won the war, but they won the peace." Countless efforts have tried to identify the factors which have contributed to their success. A recent article summarizing numerous studies concluded that Japanese companies exhibit these prominent characteristics:



1. Excellent management-employee relations, resulting in a team spirit that permeates their entire organizations.
2. Loyalty and commitment. Company for the employees and employees for the company.
3. Continuing good attendance at work. Strikes aren't nearly as common in Japan as in the United States, and absenteeism is not nearly the problem there as it is here.
4. Dedicated efforts to produce outstanding quality products.
5. Conscientious attempts to increase productivity.

Interestingly, the parallels of these attributes have been present in our company for many years. As we examine the Savannah working environment we find:

1. Excellent management-employee relations culminating in a team spirit credited with numerous accomplishments which have included the development of the world's most productive sugar refineries.
2. Loyalty and commitment from the company which has resulted in each person being placed on salary and becoming a stockholder by virtue of company granted shares. The response has been loyalty and commitment from the employees who have, for the most part, made Savannah their entire working career.
3. Continued good attendance at work, a record which has remained for years significantly below the national average for absenteeism.
4. Dedicated efforts to produce products of outstanding quality, as demonstrated by the testimonies of many customers.
5. Conscientious attempts to increase productivity, as evidenced by everything discussed in this paper.

Today, with threats to our survival, Savannah is optimistic about the future of sugar. There is no spirit of pessimism within our ranks. We know that stiff competition is unavoidable. However, we will not be intimidated or discouraged. We believe, as expressed by the words of Emerson (Van Doren, 1943), that, "This time, like all times, is a very good one if we but know what to do with it." Therefore, our response to adversity is to take the offensive and do the necessary to assure our ongoing success. We know that - our PEOPLE, our PRODUCTIVITY, our PHILOSOPHIES, our PRINCIPLES, our PROCESS, our PROGRESS, and hence, our PRODUCTS are PHENOMENAL!

Thus, we're confident that the one which will continue to be PREEMINENT in the sweeteners' market will be the one that has been most PREFERRED, your PRODUCT and ours - SUGAR!

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1946. The Portable Emerson. A Comprehensive One Volume Edition of Essays, Poems, Journals, and Letters, Viking Press, NY, p. 43.

#### DISCUSSION

Question: In the planning stages of this process, did you bring in the operators, and how did you handle that?

Priester: We do obtain the input of our operators. I emphasize the extremely good relations that we have between our management and our people. Certainly they have a big input into the decisions that we made. They help us, and we work together.

## THE USE OF AN INFRARED DRYER IN DETERMINING SOFT SUGAR MOISTURES

Fred S. Goodrow

Amstar Sugar Corporation

### INTRODUCTION

Residual moisture in soft sugar plays an important role in the packagability of the product. Sugar that is too wet, or too dry, will quickly cause problems at the packaging machine. Determination of moisture by the traditional vacuum oven method requires about five hours to complete, rendering the test ineffective for control of soft sugar moisture at the point of production. A rapid, relatively simple method for determining soft sugar moisture was developed in order that moisture in the product could be controlled at the point of manufacture. Reproducibility and accuracy of the method were found to be acceptable for process control purposes.

For the purpose of this paper, the term "moisture" is intended to be synonymous with "loss on drying".

The traditional method for determining moisture in soft sugar at the Chalmette Refinery calls for drying a 4.0 g sample of soft sugar in a vacuum oven at 70°C under at least 25 inches of vacuum for four hours. Total time required for the test, including set-up, oven time, cooling, reweighing, and performing the calculations is about five hours. Because of the length of the test, it is not practical to use it to monitor and control soft sugar moisture at the point in the manufacturing process where moisture can be influenced.

Several alternatives are available for determining moisture in a variety of products. The instrumentation chosen for development consisted of a Mettler LP-16 Infrared Dryer, mated to a Mettler PM200 Balance. The instrument offers several options for automated measurement of moisture/solids. All calculations are handled by the instrument, making the instrument ideal for non-technical operators.

All parameters necessary to determine moistures are fully selectable on the instrument. Once the parameters of time, temperature, and mode of readout (either percent solids or percent moisture) are selected, the operator simply prepares a sample, places it on the balance, and starts the instrument. He is then free to see to other duties. When the test is completed, the

display retains the moisture reading until it is cleared by the operator.

Since a procedure for soft sugars was not available for this instrument, development of a suitable sample size, drying temperature, and drying time were required.

## EXPERIMENTAL AND RESULTS

### Temperature

Preliminary tests were run on various soft sugars to determine the rate at which the sugar achieved constant weight, signifying that all of the moisture had been driven off. The instrument is capable of drying samples at any temperature in the range of 50°C to 160°C, in 5°C increments. Temperatures from 90°C to 150°C were tried, at a fixed drying time of 10 minutes. Moistures reported at 100 to 120°C agreed fairly well, but beyond 120°C, additional weight losses were reported that may have been due to other factors. Below 90°C, moistures failed to equal the splits run in the vacuum oven. Figure 1 illustrates these points.

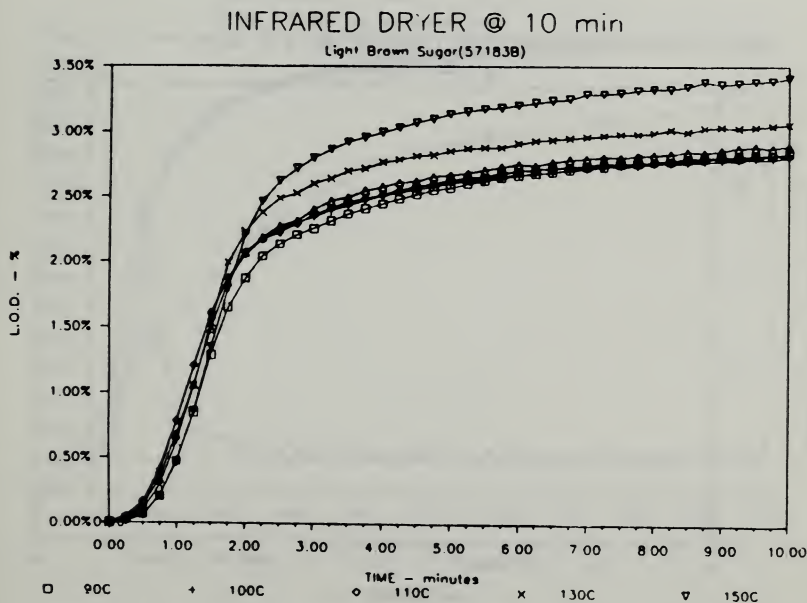


Figure 1.--Effect of temperature on weight loss (L.O.D.) in a light brown sugar in the Mettler Infrared Dryer.

As a secondary check, colors were run on the sugars once they had been dried in order to determine the temperature at which darkening of the product began to appear. It was felt that darkening of the product would be indicative of oxidation, and concurrent weight change. The colors, run on an Agtron M-500A, were referenced to colors run on the vacuum oven sample. In the range of 90°C to 110°C, the darkening had no effect on the final moisture of the sample. Color vs temperature data is presented graphically in Figure 2.

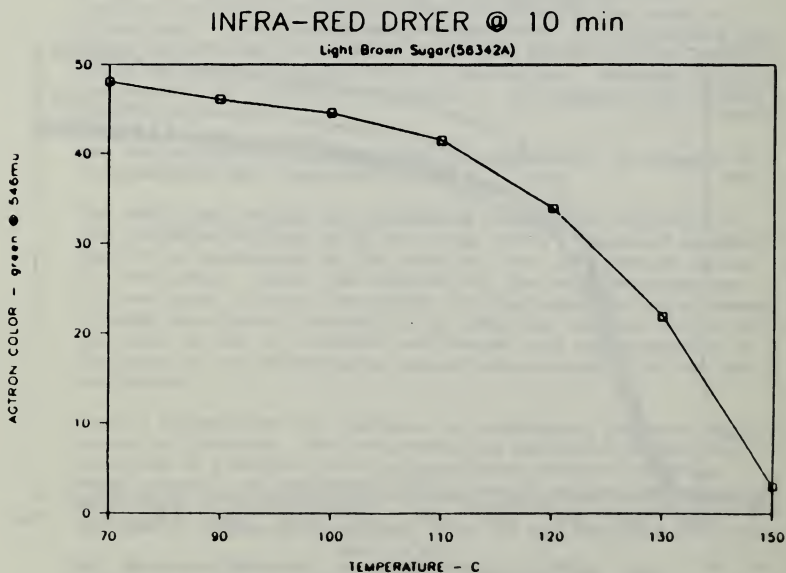


Figure 2.--Effect of temperature of drying on Agtron color of a light brown sugar heated for 10 min. in the Mettler Infrared Dryer.



Vacuum oven moistures were run on splits of the samples tested, and it was found that infrared moistures run at 100°C most closely matched the values derived from the vacuum oven method.

A temperature of 100°C was chosen as the standard temperature for control work.

### Time

Soft sugars were dried at 100°C for 20 minutes to characterize weight loss as a function of time. Most of the weight change occurred within the first 8-10 minutes, as illustrated in Figure 3. Infrared moistures run for 10 minutes most closely approximated moistures by the vacuum oven method. One of the primary considerations in developing this method was to keep the drying time to a minimum, in order that the information generated be useful in setting up centrifugal spin times.

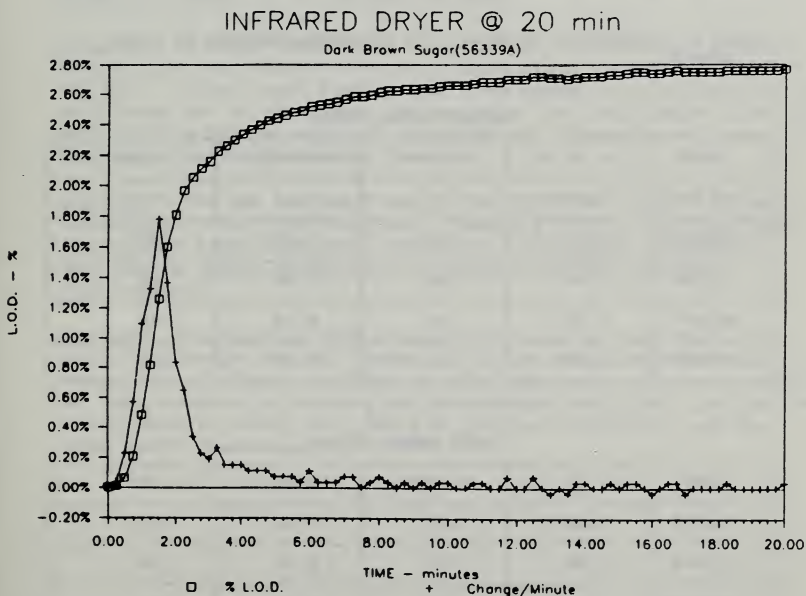


Figure 3.--Effect of time on moisture loss (L.O.D.) of dark brown sugar dried in Mettler Infrared Dryer.

### Sample size

The Infrared instrument accepts an aluminum sample dish, approximately 95 mm in diameter by 7 mm deep. Ten grams of soft sugar was chosen as the standard sample size because it covered the entire diameter of the sample dish, and it allowed repeatable measurements. Samples less than 10 grams began to exhibit reproducibility errors, as did samples that were packed too tightly in the sample dish.

Once the optimum sample size was chosen, and the time and temperatures were selected, representative samples of typical soft sugars were analyzed with the infrared dryer, and a split of the sample was analyzed by vacuum oven. All samples were run in triplicate. The data in Table 1 summarizes the findings. Moistures in the L.O.D. column were those generated by the vacuum oven method, and the moistures in the Infrared column were derived from the Mettler device. Standard deviations are listed for each set of samples.

Table 1. Comparison of methods to determine moisture in soft sugars.

#### **LIGHT BROWN SUGAR**

CODE	L. O. D.	S. DEV.	INFRARED	S. DEV.
57176A	2.64 %	.11	2.61 %	.09
57182A	2.05	.02	2.03	.02
57183A	2.39	.02	2.38	.03
56282A	2.28	.01	2.28	.01
57183B	2.83	.04	2.82	.05

#### **DARK BROWN SUGAR**

CODE	L. O. D.	S. DEV.	INFRARED	S. DEV.
56339A	2.60 %	.01	2.62 %	.01
56342A	2.53	.01	2.50	.05
556352A	2.48	.01	2.48	.02
57093A	2.54	.02	2.52	.02
57104A	2.53	.01	2.52	.02

## CONCLUSION

Moistures run on the Infrared balance were comparable to moistures derived from the vacuum oven method. The Infrared balance is easily used by non-technical personnel, and it can yield accurate results in far less time than is required by the vacuum oven method.

## ACKNOWLEDGEMENTS

The author would like to express his appreciation to Mr. Armando Abay, Refinery Manager of the Chalmette Refinery, and Dr. Chung C. Chou, Technical Manager, Amstar Sugar Corporation for their help and guidance in this project.

## DISCUSSION

Question: What is the cost of the instrument, and is it rugged enough to be put in the refinery itself?

Goodrow: It costs about \$3800, and, no, it is not rugged enough to be put into the refinery because it does use a 3-place electronic balance. If you could keep it away from humidity and vibrations, it would work out in the plant. In our case, our centrifugal floor is only about a half minute walk away from the laboratory so we are keeping it in the laboratory for now.

Question: You are obviously making two different colored products to specific color specifications. Assuming there is no consistent correlation between moisture and color, which takes precedence--moisture to ensure packability or color to satisfy customer requirements?

Goodrow: We basically make our sugars to a color initially, but there is a range that is allowed within our color specifications to where we can, within the limits of the color specs, choose a higher or lower moisture by setting out centrifugal spin time. So, yes, we always operate within the limits of a color constraint, and the color takes precedence.

## CRYSTALLIZATION STUDIES IN LOW GRADE SYRUP - UPDATE

Raymond E. Dickey and Joseph F. Dowling, Refined Sugars Inc.

### INTRODUCTION

In an effort to determine the effect of crystal number on sucrose exhaustion from low grade syrup, a study was undertaken at Refined Sugars Inc. in 1986.

The study at that time was done in conjunction with Tate & Lyle Group Research and Development, Reading, U. K. and the results were presented (Dickey et al., 1988).

The purpose of this report is to apply data from a modified three boiling system to the regression formula developed at that time and so to determine if any variation from previous findings is evident.

To accomplish this, several parameters such as "g", massecuite purity, crystal volume and number, invert content and axial ratio were applied to the formula and the variance examined.

### EQUIPMENT DESCRIPTION AND CRYSTAL MEASUREMENT

The equipment and measurement technique have been described in the aforementioned paper but will be reviewed here for the purpose of clarity.

The method of crystal measurement employs a VIDs system with ancillary D.O.S. software. The software runs on the Apple II microcomputer equipped with a disc drive and an Apple graphics tablet with a printer interfaced internally.

A video camera and green video monitor are also interfaced with the microcomputer. Using these in conjunction with the graphics tablet makes it possible to measure the size of the crystals on the projected slide image by employing a light pen.

The slide is prepared by forming a slurry of the massecuite in question with invert syrup at about 76% solids.

A detailed description of the crystal sizing is contained in the previous paper and so will not be repeated here. After measurement of 200 crystals, the program displays a menu which allows sizing calculations to be performed.



Data for this paper is based upon sizings for crystal volume and axial ratio, i.e. b axis/ c axis.

When a particular file is called up and the corresponding instruction given, the computer performs a sizing of the crystals in memory and prints out a distribution of the size ranges as well as an average size of the crystals measured. A typical data sheet showing distribution bands and average size for crystal volume is shown in the previous paper (Dickey et al., 1988).

#### ANALYSIS OF THE MASSECUITE

The procedure for massecuite analysis is contained in the previous paper but will be restated here for clarity and continuity.

To determine what the variables were in a particular massecuite, certain analyses were necessary. Prior to measurement of the crystals, the massecuite was analyzed for solids and apparent purity. The massecuite was then passed through a vacuum filter containing a screen of 50 mesh to remove the crystals. This was followed by a determination of purity on the molasses filtrate.

By applying SJM formula, the percent crystals in the total massecuite could be determined. By knowing the total volume of the "C" pan, the pounds of crystals could be calculated. The remelt pan for boiling "C" strikes was estimated to contain 39.64m<sup>3</sup> of massecuite when filled to capacity. Since the specific gravity may be stated as 1.5 tons/m<sup>3</sup>,

$$\text{m}^3 \text{ "C" Mass} \times \% \text{ Crystals} \times 1.5 = \text{Crystal Tons}$$

$$\text{Crystal Tons} \times 2240 = \text{Crystal Pounds}$$

As stated above, a full capacity pan contains 39.64m<sup>3</sup> of massecuite,

$$39.64 \times \% \text{ Crystals} = \text{Total Crystal Volume}$$

Now from the measurement data of 200 crystals, the average volume of a crystal (mm<sup>3</sup>) was calculated. Therefore, it was now possible to calculate the total number of crystals per strike.

$$\frac{\text{Total Crystal Volume}}{\text{Single Crystal Volume}} = \text{Total Crystal Number}$$



## DATA EXAMINATION

### (1) Initial Study - Two Boiling System

In the previous study at Refined Sugars, which was reported to S.P.R.I. in 1986, our analyses enabled us to determine the effect of twelve variables on molasses exhaustion. These variables included "g", massecuite purity (P), crystal number, crystal volume and axial ratio.

The molasses exhaustion was measured in terms of the difference between actual and ideal total sugars and was calculated according to the following formula:

$$\text{Plus Ideal} = \text{Total Sugars} - \frac{100 (5 + 3g)}{3 (3 + g)} \quad (1)$$

The previous study included 370 strikes and a multiple regression analysis on these strikes by Tate & Lyle Group Research and Development at Reading, U.K. resulted in the following regression equation:

$$\begin{aligned} \text{Plus Ideal} &= 3.66 g + 0.15P - K \\ \text{where "g"} &= \text{Invert/Non-Sugars} \\ P &= \text{Massecuite Purity} \\ K &= \text{Constant} \end{aligned} \quad (2)$$

It can be seen from the formula that the only variables which affected exhaustion were "g" and massecuite purity.

### (2) Present Study - Three Boiling System

The data presented for this paper reflects the results obtained from a modified three boiling system and relates to a study of 735 strikes. We did not determine a multiple regression analysis on the new data to obtain a new regression equation, but rather applied the data obtained from the strikes to our previous "Plus Ideal" equation to see if there was any change in the effect of the variables examined on sucrose exhaustion.

Application of the data to the previous "Plus Ideal" equation appeared to confirm the fact that of the variables examined, only "g" and massecuite purity had an effect on sucrose exhaustion with "g" having the greatest overall effect.

## ANALYSIS OF VARIABLES

### Effect of "g"

Graph I shows two sets of data points which reflect the effect of increading "g" to "plus ideal" performance. The "plus ideal" numbers on the ordinate axis is the actual ideal from the data with a constant subtracted.

The upper trend line reflects the effect of "g" with a two boiling system and the lower line the effect of "g" with a modified three boiling system.

Examination of the two lines shows that for similar values of "g", the "plus ideal" values are lower for the modified three boiling system. This may be attributed to the fact that for that system the massecuite from which the final molasses was boiled had lower purity throughout the study.

In addition, for the two boiling study, data points for a "g" value of 0.9 or lower totaled 110, or 32% of the strikes while for the three boiling study the corresponding total number increased to 363 or 50% of the strikes.

The larger amount of strikes at low "g" value for the three boiling study should give more validity to the data in that range, which reflects a changing slope in the three boiling line.

### Effect of Massecuite Purity

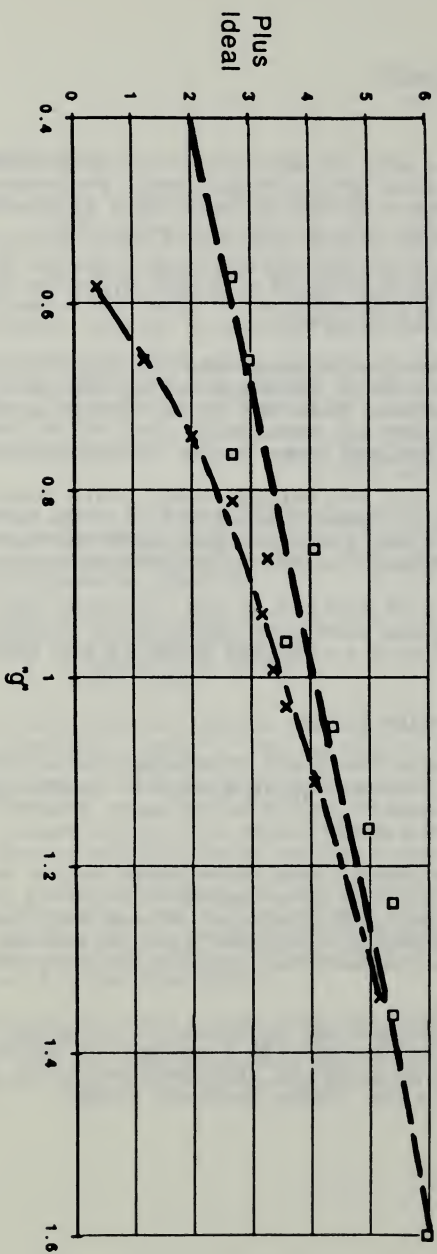
Graph II represents the plots of average data points for each of the boiling systems studied with respect to the increase of massecuite purity to molasses exhaustion performance ("plus ideal").

By examining the trend lines, two things become readily apparent. Firstly, the lower massecuite purity produced by the three modified boiling system reduced the "plus ideal" as expected. Secondly, the lower line reflecting average data points from the modified three boiling system showed less scatter.

The fact that less scatter or better correlation is viewed about the lower "three boiling line" may be due to the larger number of data points and lower values of massecuite purities found in the "three boiling" study.

# GRAPH I

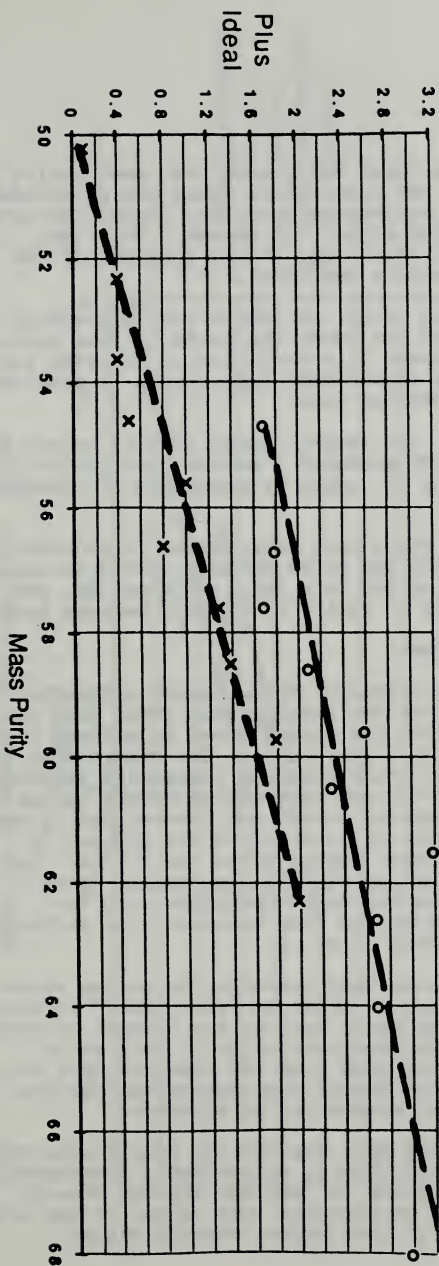
Graph Showing the Effect of Plus Ideal to Increasing "g"



Note: Each point represents 50-100 strikes

# GRAPH II

Graph Showing the Relationship of  
Plus Ideal To Mass Purity



Note: Each point represents 50-100 strikes



A comparison of "plus ideal difference" vs. mass purity is shown in Graph III. The theoretical ideal was calculated using the plus ideal regression equation, fixing the mass purity at 60, and using actual "g" values. This then eliminates the effect of "g" and should show better the correlation between purity and ideal.

Examination of the two lines, the upper line reflecting the two boiling system and the lower the three boiling system, shows that an improvement in correlation is observed for the upper line but no significant improvement is observed for the lower three boiling line.

As stated previously, the larger number of data points for the three boiling study produced a better correlation of increasing mass purity vs. ideal irrespective of changing "g".

In summary the graph shows that improvement in sucrose exhaustion for the modified three boiling system correlates well with massecuite purity to a value of about 54, and with further reduction in purity the effect becomes less.

#### Effect of Crystal Volume

The effect of crystal volume on "plus ideal" difference is calculated by subtracting the theoretical ideal from the regression equation from the actual ideal calculated from the Tate & Lyle formula (equation 1). The theoretical ideal is the predicted ideal from the regression equation which compensates for "g" and mass purity effect (equation 2). The difference between actual and theoretical is shown in Graph IV where groups of data points are plotted in order of ascending crystal volume along the x axis. In Graph IV the "zero line" represents the average "plus ideal" predicted by the regression equation, and the difference above or below the line represent the difference of the actual "plus ideal" from it.

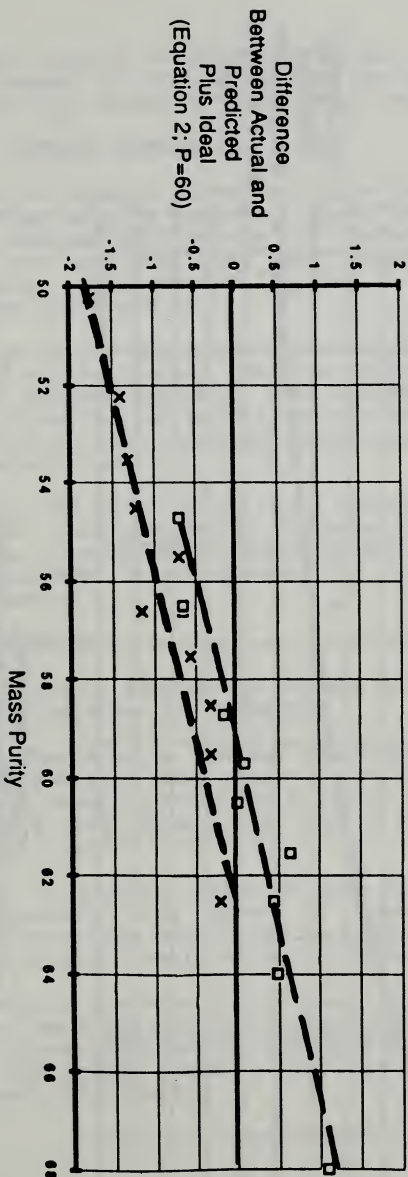
Looking at the trend line drawn through the points shows that the effect of crystal volume on "plus ideal" is slight with the largest volume point showing the largest decrease in "plus ideal" (or the best exhaustion). This would appear to contradict findings from the previous data on the two boiling system which showed that the largest crystal volume gave the poorest exhaustion performance.

However, it may be noted that the average data points which show the highest average crystal volume had a corresponding lowest "g" value, and since "g" has the highest overall effect on "plus ideal" performance, the slope of the line is more due to lower "g" than higher crystal volume.



GRAPH III

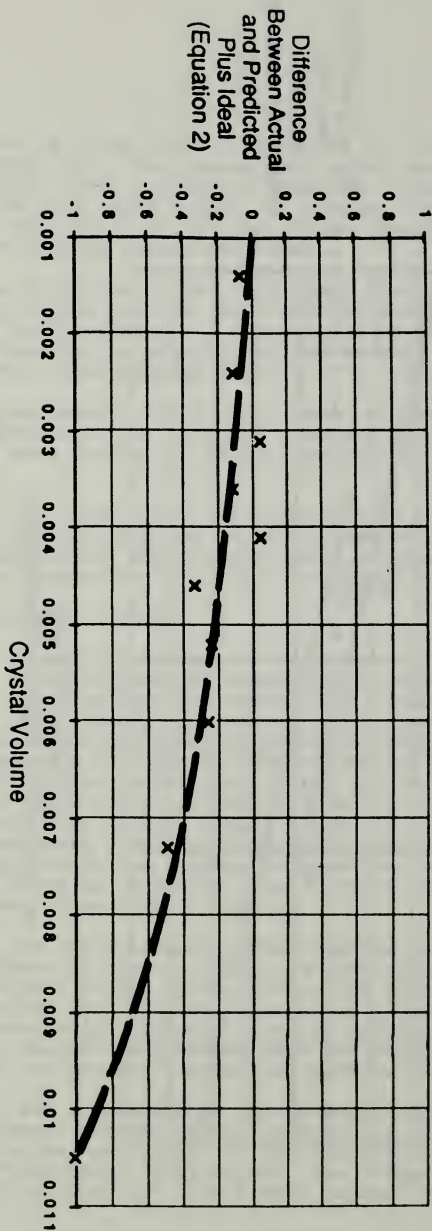
Graph Showing the Effect of Mass Purity on  
"Plus Ideal Difference"



Note: Each point represents 50-100 strikes

# GRAPH IV

Graph Showing Increasing Crystal Volume to  
"Plus Ideal Difference"



Note: Each point represents 50-100 strikes

The largest crystal volume occurred for a sample population with the lowest "g" indicating that the most rapid growth occurred at the lowest "g" and invert values.

#### EFFECT OF CRYSTAL NUMBER

The effect of crystal number on "plus ideal difference" for the three boiling system is shown in Graph V which plots the average data points for increasing crystal number along the "x" axis with plus ideal difference plotted on the "y" axis. Plus ideal difference is calculated the same way as done for Graph IV.

Examination of the graph shows that when a crystal number of  $15 \times 10^{-11}$  is reached that the effect on "plus ideal difference" becomes minimal. This agrees reasonably well with data from the two boiling system which concluded that a minimum of  $10 \times 10^{-11}$  crystals is required to maximize sucrose exhaustion.

The area of the graph which shows improved "plus ideal performance" (the area below the zero line) contains sample populations corresponding to the lowest "g" and mass purity values which very likely account for the slight improvement in performance.

#### EFFECT OF INVERT

The effect of increasing percent invert on "plus ideal difference" is shown in Graph VI.

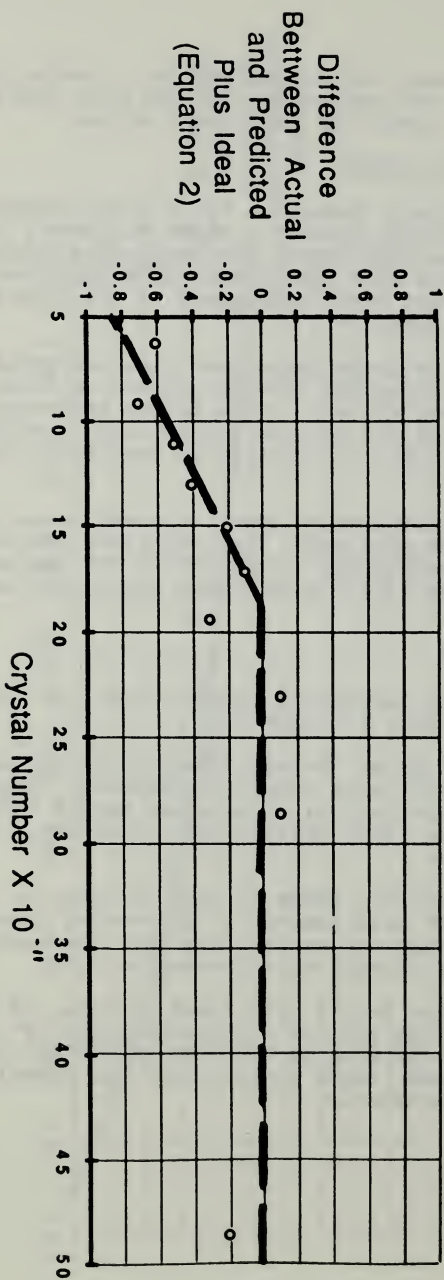
There should be an obvious relationship between the invert percent and "plus ideal" performance. Since an increase in invert content of molasses should raise the total sugars, the "plus ideal" should increase. This was found to be true.

Graph VI however, shows no such increase in "plus ideal difference" with respect to increasing invert which is almost certainly due to the fact that increasing invert should correlate closely with increasing "g".

Since both the Tate & Lyle ideal formula and the R.S.I. regression equation contain a correction for "g" it appears that the close adherence of the data points to the "zero line" of the graph, tends to show that the formulae show a rather good relationship.

# GRAPH V

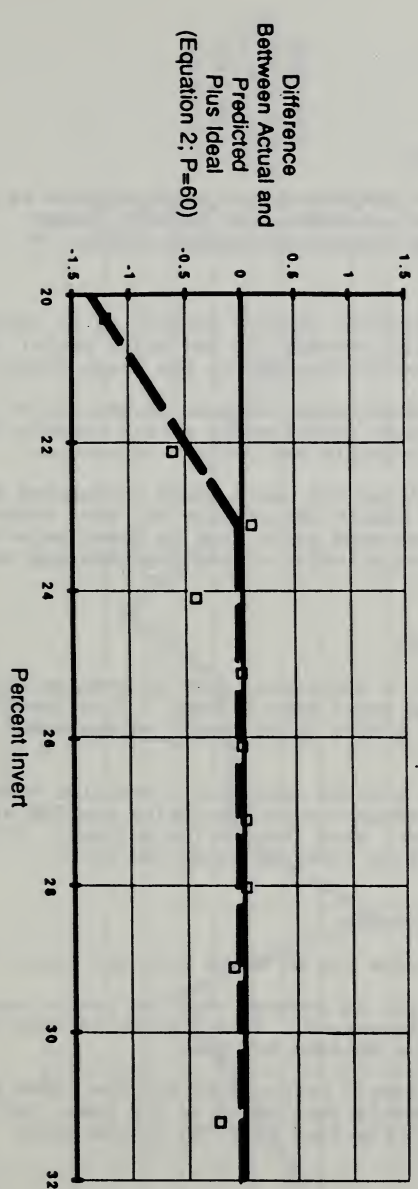
Graph Showing the Effect of Crystal Number to  
"Plus Ideal Difference"



Note: Each point represents 50-100 strikes

GRAPH VI

Graph Showing the Effect of Percent Invent on  
 "Plus Ideal Difference"



Note: Each point represents 50-100 strikes



## Effect of Axial Ratio

As stated in the previous paper which related to the two boiling study, it is known that dextran causes elongation along the c axis (Sutherland, 1968).

Since dextran inhibits sucrose exhaustion it follows that with high dextran content (or low axial ratio) the sucrose exhaustion would diminish in low grade syrups.

The conclusion drawn from the data of the two boiling study was that no clear relationship exists between the axial ratio of the crystals and sucrose exhaustion.

Examination of Graph VII which plots increasing axial ratio against "plus ideal" performance for the three boiling system, shows once again that no clear relationship exists between the axial ratio of the crystals and sucrose exhaustion.

## Data for Total Sugars

Graph VIII shows a historical plot of average plus ideal values for the years 1980 - 1988. "Plus ideal" shown on the chart is the actual "plus ideal" values minus a constant.

The chart shows that by employing a modified three boiling system to reduce massecuite purity coupled with a minimization of invert gain through the refinery, it was possible to reduce plus ideal by about two units.

## CONCLUSION FROM THE DATA

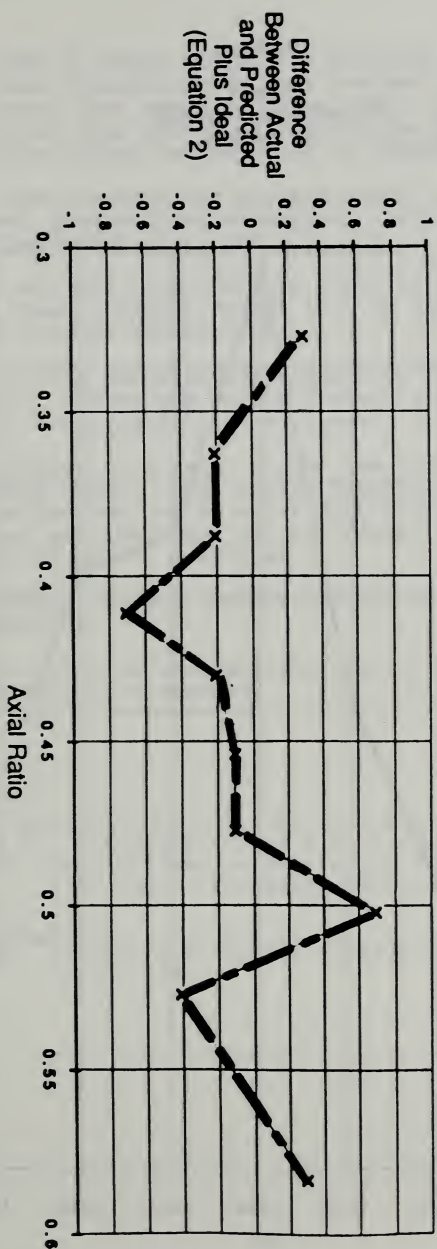
Several conclusions can be drawn from the data:

1. Sucrose exhaustion is reduced when the invert/non-sugar ratio ("g") is increased. This applies to both the two boiling and three boiling systems.

Superior "plus ideal" performance for the three boiling system can be partly attributed to the lower "g" values which relates to the fact that "g" is the major contributor.

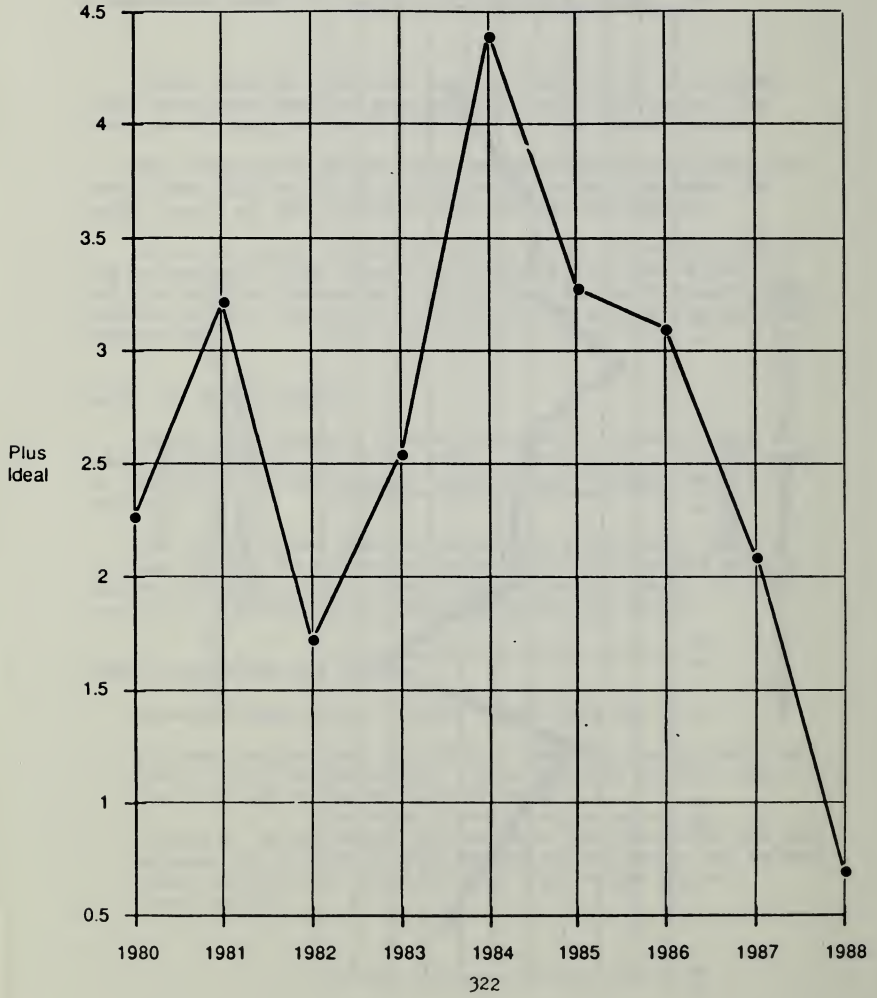
# GRAPH VII

Graph Showing the Effect of Axial Ratio or  
"Plus Ideal Difference"



Note: Each point represents 50-100 strikes

GRAPH VIII  
Yonkers  
Total Sugars Plus Ideal  
on Molasses



2. Boiling at lower massecuite purities produces better sucrose exhaustion for both two and three boiling systems. The superior "plus ideal" performance for the three boiling system was due to the fact that a three boiling system led to lower "C" massecuite purities.
3. For the previous two boiling study it was found that to effect good sucrose exhaustion a minimum crystal number was necessary (viz.)  $10 \times 10^{-11}$ .

This was not found for the three boiling study where the lowest crystal number ( $7 \times 10^{-11}$ ) produced the best sucrose exhaustion.

The reason for superior exhaustion performance at low crystal number may be attributed to the low "g" values which corresponded to low numbers of crystals.

4. The effect of crystal volume was less for the three boiling study. Once again, crystal volume effect was minimized due to the overriding effect of low "g" values and the small ranges encountered for the study (viz.) 0.0015 - 0.0075 mm<sup>3</sup>.
5. Increasing invert content did not diminish sucrose exhaustion.
6. Axial ratio of the crystals had no effect on sucrose exhaustion performance.

## REFERENCES

- Dickey, R.E., J.F. Dowling and R.M. Morton.  
 1988. Crystallization studies in low grade syrup. Proc. 1986 Sugar Processing Research Conf., pp. 34-53.
- Sutherland, D.N.  
 1968. Dextran and crystal elongation. Internat. Sugar J., 70:355-358.

## DISCUSSION

Question: I am not sure that it is the dextran that causes crystal elongation. We pointed out yesterday that, although dextran is accused of this, it is not really at fault.

Dickey: I understand from what you and Earl Roberts and others have indicated, if pure dextran is put into pure sucrose, no elongation of the crystal along the C-axis takes place. That is pretty well established. There are probably other organic nonsugars or ash constituents or even combinations of these that are responsible for elongation. I am going on the assumption that dextran is correlated to crystal elongation even if not solely responsible for it.

Question: You are looking at crystal volume and number of crystals. Have you tried looking at total crystal surface area?

Dickey: When we first looked at this, we made the assumption that we needed a certain number of crystals to effect good sucrose deposition. When talking about total surface area, you would assume that if you had a smaller crystal volume for the same crystal number, you have a greater surface area, so we try to incorporate that. However, we did not specifically calculate the surface area. We made the assumptions I just mentioned about the relationship between crystal volume, number and surface area.



## UTILIZATION OF LASER OPTIC TECHNOLOGY TO DETERMINE CRYSTAL SIZE

Mark E. Holle

American Crystal Sugar Company

### INTRODUCTION

Several commercial offerings of a new type of particle size analysis offer methods made possible by solid state lasers. This paper represents some experience with the LASENTEC LAB-TECH 100. It also includes some attempts to compare data provided by this methodology to those statistics historically used to estimate these data, namely screen test analysis.

### PRINCIPLE OF OPERATION

This device measures a number of particles by actually scanning a beam of focused, coherent laser light across individual crystals at a known rate. These crystals are held in liquid suspension and move past the focal point by flow entrainment. Analyzing backscattered light allows calculation of the distance on the particle the spot traveled and accumulations of these distances contain the particle size information we seek.

The laser used is a low power, solid state laser emitting in the visible red region. Spot size with this system is about 2 microns. This is also about the order of minimum measurable particle size.

Optical elements are mechanically driven to scan the focal point through the suspending solution to strike particles causing light to backscatter for the duration of the coincidence. The LAB-TEC we tested had proprietary discrimination which measures that backscattered light at two places; equally spaced from the incident beam. This allows rejection of particles out of the focal point. The mechanical scanning moves the focal point in a small angle sweeping a distance at the focal point on the order of mils (fig 1). This is probably near the order of the largest particles which can be included in the measurement.

Since scanning frequencies were in the high audible range it is likely that scanning velocities were:

Scanning velocity  $\sim 10^{-3} \text{ m} / 10^{-3} \text{ s} \sim 1 \text{ meter/sec.}$

With scanning velocities on the order of meters/sec we expect that particle velocities relative to the scan would have to be much smaller to be negligible. Moving the beam perpendicular to the particle flow rather than parallel to it results in data which is largely unaffected by particle flow speed.

After discrimination further electronic hardware sorts the accepted backscattered times to counters by time. In the model we tested eight channels ( $n=0 - 7$ ) of counts were available. In addition a programmable time scale (T) provide time proportional calibration and a hardware range selector allow coarse range selection. ( $R=1000, 500, 250$ , or  $125$  for the -XS model). Times were classified with standard screen scale increments (forth root of two series) though other scales are available. It can be seen that the range selections provide some overlapping of class sizes between ranges and from the raw data example of Table 1. Table 2 shows how the ranges are derived.

example:  $T=1, R=1000$

Channel	$n=0$	$t > T \cdot R \cdot 2^{(-n/4)}$	$t > 1000$ microns
	$n=1$	$T \cdot R \cdot 2^{(-n/4)} < t < T \cdot R \cdot 2^{((-n+1)/4)}$	$841 < t < 1000$
	$n=2$	"	$707 < t < 841$
	$n=3$	"	$595 < t < 707$
	$n=4$	"	$500 < t < 595$
	$n=5$	"	$420 < t < 500$
	$n=6$	"	$354 < t < 420$
	$n=7$	$t < T \cdot R \cdot 2^{(-n/4)}$	$t < 420$

Table 2. How class limits are established for the LAB-TEC 100-XS. Other LASENTEC offerings allow other range and division selections including binary (power of two) and equal (linear) increments.

In addition to access to the counts which are accumulated in these counters over a selected time interval, the instrument is provided with a small micro-computer which allows a number of built-in numerical manipulations and the output of bar graphs of particle size distribution. The primary manipulation which the operator has direct control over is a weighting vector which allows a coefficient for each count. It is suggested that these be manipulated to correspond to an accepted standard. A form factor manipulation subtracts from the class data in a manner proportional to class size, and it is suggested that this can usually accommodate particles of unusual shape. The LABTEC 100 provides output to a built in liquid crystal display and to a printer.

LASENTEC Particle Size Analyzer Experimental Data  
Particles Counted

Class	<	44	53	62	74	88	105	125	149	177	210	250	297	354	420	500	595	707	841	>	Total
Sample																					
d147	34848														418	285	165	112	56	26	11 35921
c147	32155																				309 35714
b147	28226																				1911 35917
a147	23472	1400	1369	1226	1271	1162	1116														5480 36496
d175	36022																				11 36971
c175	33141																				288 36484
b175	29405																				1776 36667
a175	24007	1421	1403	1215	1277	1137	1081														4883 36424
d208	32602																				9 33438
c208	30579																				252 33397
b208	27401																				1504 33397
a208	22790	1266	1189	1078	1085	938	913														3951 33210
d246	24203																				43 25392
c246	22331																				463 25200
b246	19946																				1851 25300
a246	16913	808	768	696	747	689	661														3848 25130
d295	22732																				65 24056
c295	20861																				559 23688
b295	18550																				2023 23863
a295	15915	775	748	640	695	645	658														3958 24034
d351	23379																				72 24699
c351	20459																				564 23062
b351	19003																				1928 24287
a351	16277	791	769	672	692	657	634														3780 24272
d417	21609																				77 22663
c417	20314																				542 22581
b417	18052																				1455 22254
a417	15315	691	656	590	633	550	519														2965 21919
d495	22800																				92 23924
c495	21810																				578 24251
b495	18777																				1536 23279
a495	16340	828	825	744	779	658	606														3134 23914
d589	20939																				94 21864
c589	19815																				499 21713
b589	17373																				1218 21007
a589	14553	701	686	603	622	580	495														2497 20737
d701	21997																				62 22742
c701	21873																				438 23499
b701	20643																				1114 23957
a701	17725	675	619	548	583	498	435														2132 23215
d-all	22918																				40 23933
c-all	21374																				428 23586
b-all	17444																				1141 20863
a-all	15677	696	624	526	536	498	459														2534 21550

Range Selection: d-1000 c-500 b-250 a-125

Table 1

## APPLICATION IN THE SUGAR INDUSTRY

### Dry Granulated Sucrose

It would be nice to have an accurate test which could be applied with a minimal amount of time and expense to determine crystal size and size distribution in final product. A single test which gives counts in 8 channels takes less than a minute to run. Sample preparation concentration is not critical; we used a 2% by weight amount of dry powder in about 80 ml of suspending liquid. We tried several suspending solutions including oils and alcohols as suspending fluid. Salad oil and mineral oil work but tend to entrain air. Methanol seemed to work better in this regard and was used in most of our work. Among factors to consider in solution selection are: refractive index, viscosity, insoluble to sucrose, expense, and use hazards.

More critical is the focus adjustment which must be carefully located just inside the beaker wall, a focal point too deep in the solution becomes occluded by a "snow storm" effect of particles passing between it and the backscatter detectors. Once adjusted numerous samples could be run without readjustment.

It was noted that a continuous reduction in sizes would result from testing the same sample over a prolonged period. This effect was due to the magnetic stirring bar grinding up the crystals; the effect was significant even during usual sample times. Replacement of the instruments stirring mechanism with an overhead stirring impeller eliminated the problem.

Data from crystalline sugar products tends to always have a very large number of counts in very smallest size category. To some extent this is a property of the method and we would expect that beam intersections with the corners of particles could produce counts which could not be discriminated against. Contributions from such occurrences are statistically repeatable with high precision though they limit the ability of the instrument to provide distribution information. Further, the sugar crystals provide brief flash of light each time an edge between two crystal facets is encountered by the focal point and these pass discrimination and accumulate in the smallest time class counter which has no lower limit. Because of this our analysis of the data generally just ignores this class because the information is mostly noise. This effect was not so prevalent in non-sucrose samples. A given sized sucrose crystal has facets of various dimensions and any of these facets may be traversed (even diagonally) providing many possible lengths for a single sized crystal. Also it is probable that only facets of the crystals which are nearly normal to the incident beam backscatter in a fashion which passes discrimination. Because of this distribution information for sucrose may be very difficult to surmise from the data produced by this method.



## Experiment: A Composite Sugar and Its Fractions

An experiment that provided some insight into the devices usefulness was one in which a composite of our Moorhead plant production over a campaign was analyzed on the LABTEC 100. That same sample was fractionated using a screen test analysis. In addition the fractions retained on each screen, except the first and last which proved to small to sample, were run on the LAB-TEC 100 (Table 1). Each sample was run on each of the devices four ranges (R=1000, 500, 250, and 125) though it is probable that mean size information can be discerned from an analysis on a single range. It is useful to note how consistently the instrument overlaps ranges.

In the graphical representation of the screen test (Fig. 1) data was compressed to eight screens by adding least significant screens together. A histogram, bargraph, or line representation of a screen test are all useful in conveying both mean, or central tendency, and variation, or distribution of sizes.

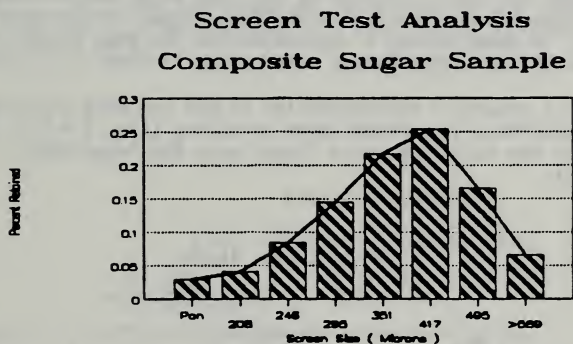


Figure 1

One of the suggested ways of viewing the output of the laser based device was to apply a weighting coefficient to each class that yields the same results as an accepted means of sizing. Coefficients were calculated which do this and are used in the comparison of Fig. 2.



## Comparing Composite With Large & Small Fraction

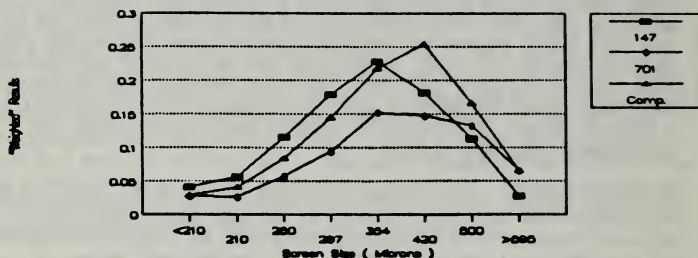


Figure 2

Here a large and small fraction of the same sugar are treated in the same manner and presented on the same graph. Using this treatment, it seems though a representation for mean seems weakly maintained, distribution information is lost.

Looking at a graphical representation of the raw data collected in this experiment is somewhat more revealing (Fig. 3 & Fig. 4) inasmuch as the data seem quite linear over the logarithmic screen scale.

## Fraction Data 147-295 & Composite

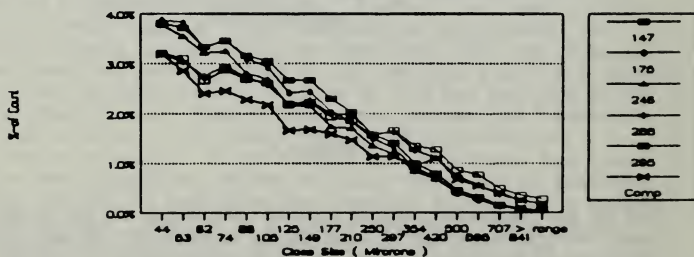


Figure 3

# Fraction Data 351-701 & Composite

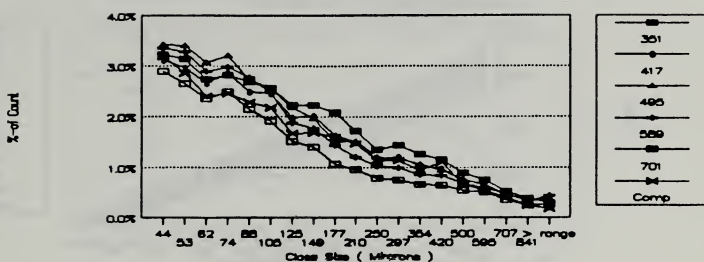


Figure 4

Linear regressions of the data (Fig. 5 & Fig. 6) seem to indicate a definite trend as the mean size of the fractions varies. Possibly the slope and the offset of the resulting regression could be combined in a function to approximate the population mean.

# Linear Fits 147-295 & Composite

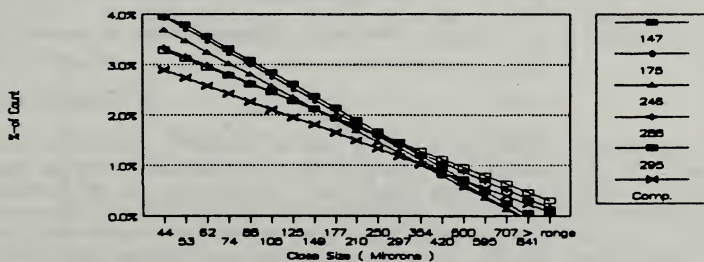


Figure 5

# Linear Fits 351-701 & Composite

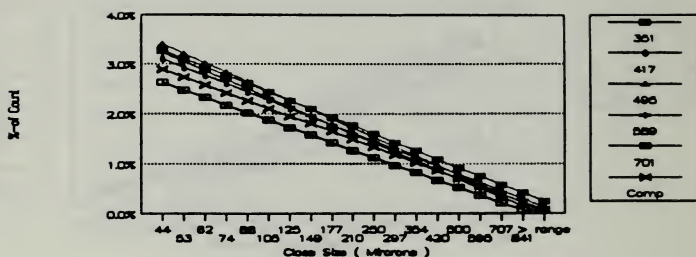


Figure 6

Examining the slope and offset of the regression equations show indications of a trend (Fig. 7 & Fig. 8) though two of the fractions, 495 and 589, don't follow the pattern. Certainly more work needs to be done if this is to be a useful method.

## Regression Slope versus Fractions

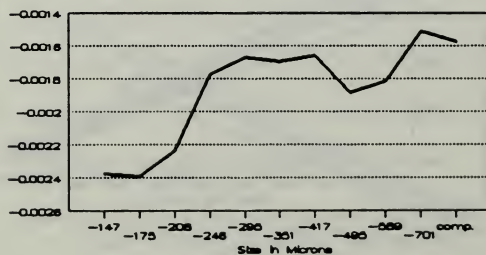


Figure 7

## Regression Constant versus Fractions

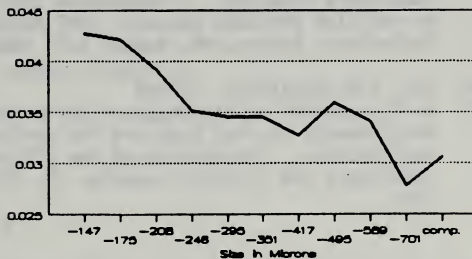


Figure 8

These results aren't too surprising, considering the shape and optical properties of individual crystals; each facet, no matter how small, can produce a sharp-edged (high rise-time) signal which passes the discrimination circuitry. Still, statistics gathered in this manner are highly reproducible (within 0.3% at 100,000 counts). Tests conducted in which larger crystals were added to a sample being run always resulted in the slope and offset of the distribution moving in the expected direction.

### ON-LINE OPPORTUNITIES

Work is proceeding on devices using this technology which can be used in online real-time environments. Knowledge of mean crystal size and rate of growth during pan boiling could be valuable. Processing delay should be under a minute, depending on how quickly an acceptable number of crystals avail themselves for counting.

### CONCLUSIONS

This technology seems to have a lot of potential, advantages such as good repeatability, quick analysis, and possible on-line application are attractive. Usefulness depends on a good understanding of the type of information this analysis provides, and this brief examination showed that direct comparison to traditional methods may not be too valuable. As the technology matures possibly discrimination techniques which take advantage of the unique optical properties of individual sucrose crystals could be devised which allow unique size assignments to each crystal examined.

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## ACKNOWLEDGEMENTS

I would like to express my thanks to Tim Haakenson who prepared the samples and ran the analysis in the experiment referred to and many others in the course of our evaluation.

## DISCUSSION

Question: You ran these in methanol; sucrose has some solubility in methanol. Did you saturate the solution before you ran it?

Holle: We noticed that the methanol would dissolve the smaller crystals at first, and after a while, the water that was in the methanol would become saturated with sugar. We ended up by filtering out all the crystals and re-using the methanol. This seemed to work quite well. We also tried a number of other solvents such as salad oil that worked reasonably well although salad oil is somewhat viscous for this purpose. There may be some other choices that might overcome that problem.



## A TEST FOR CRYSTAL QUALITY

A. V. Bailey, W.S.C. Tsang and M. A. Clarke

Sugar Processing Research, Inc.

### INTRODUCTION

The determination of yield and crystal quality in crystallizer massecuites in refineries and raw sugar factories is a constant problem. Crystal separation is at present accomplished using techniques such as vacuum filtration and the Nutsch bomb. A quick, convenient method has been devised for the separation of crystals from mother liquor (M.L.) using specially designed centrifugal filtration tubes. The tubes are suitable for analyses in the laboratory or in the plant to give data that can indicate crystal quality, yield and completeness of recovery. Other systems that can be used to study crystal quality are described by Dickey et al. (1986); and Goodacre et al. (1984). These can give much more detailed information than the tube reported herein. The value of the tube test lies in their speed and low cost. Results on tests on the accuracy, precision, and of operations of the test are presented in this paper.

### MATERIALS AND METHODS

The massecuite samples were obtained from S.P.R.I. sponsoring companies. Trials on centrifugation separations were made at the plant sites using specially designed centrifugation filtration tubes and a conventional centrifuge.

The centrifugal filtration tubes are of stainless steel construction, as shown in Figure 1, with threaded male and female segments A and B, strainer C, and splash guard D. When assembled, these elements form an airtight closed container of 0.32 cm wall thickness and ca 15 mm overall length. The strainer is 24 gauge stainless steel perforated with 0.8 mm holes drilled 4 mm between centers. The splash guard is funnel-shaped with a hole in the center, and is used to prevent the syrup or liquor from getting into the threaded union and causing sticking or "freezing" of the joint.

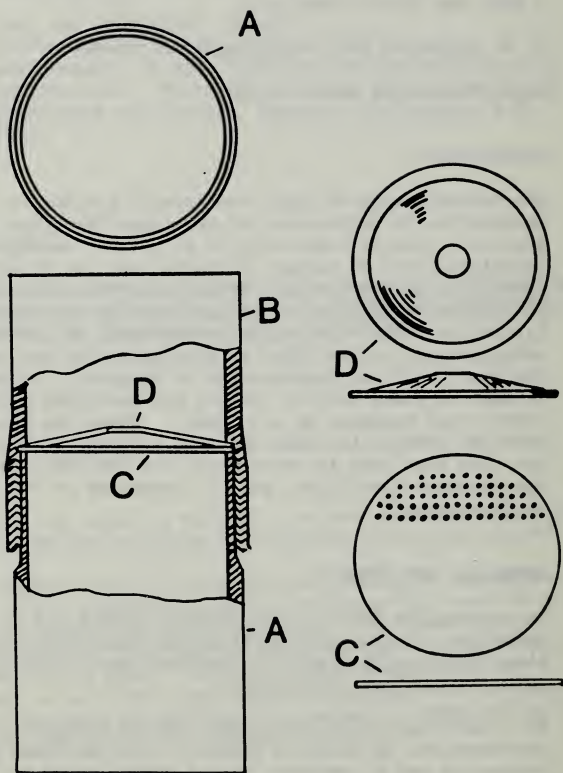


Figure 1.--Centrifugal filter: (a) crystallization chamber, (b) receiver, (c) strainer, (d) splash guard.

The massecuite is weighed directly into the male section and the device assembled. The assembly is then inverted, placed in the properly counterbalanced centrifuge cup, and centrifuged, from 5 to 30 minutes at ca 2400 rpm, depending on the size of the sample. Large samples of heavier massecuites can require extended centrifugation time. Insulation with thermal lagging material can be used to prevent cooling if desired, but, in general, this is not necessary. Disassembly can be accomplished without spilling the crystals or M.L.

The sucrose and invert sugar concentrations were determined by the method of Tsang and Clarke (1984 and 1986). Ash was determined by the ICUMSA conductivity method (Schneider, 1979).

## RESULTS AND DISCUSSION

### Effect of time on centrifugation

Fractional samples of M.L. were obtained from a 200 g massecuite sample by halting the centrifugation after 25, 50, and 75 minutes and removing the M.L. The rate of separation of the M.L. from the massecuite is illustrated in Figure 2. A 50 g sample which had been separated similarly is included for comparison. It can be seen that the separation of the 50 g sample is virtually complete in the first 30 minutes whereas that of the 200 g sample is still incomplete after 75 minutes.

The three M.L. fractions obtained from the 200 g sample were analyzed by HPLC for their sucrose and invert sugars concentrations; the results are presented in Table 1. The sucrose concentration of the M.L. fractions showed a slight decrease with centrifugation time whereas the invert sugars concentration of the three fractions was constant.

Table 1.--Mother liquor (200 g sample)<sup>1</sup>

Spin time (min)	Sucrose % HPLC	Invert sugars % HPLC	Ash %
0	73.1	4.4	--
25	53.7	8.2	10.0
50	50.4	9.0	9.5
75	48.6	8.5	9.7

<sup>1</sup> Average of three determinations

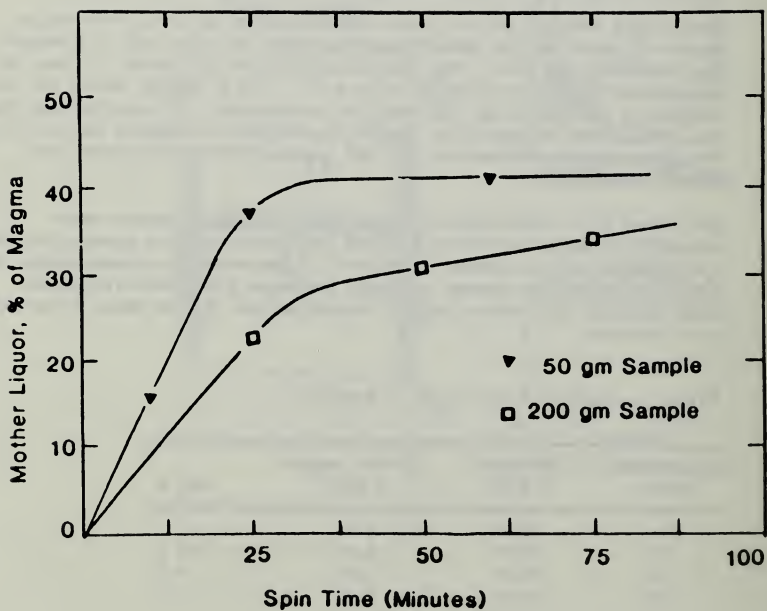


Figure 2.--Mother liquor removed.

The decrease in sucrose concentration in the relatively small amounts of M.L. collected in the last fraction is probably caused by cooling of the massecuite during the lengthy centrifugation time. The ash content, because of its low initial value, was less affected.

Using 50 g samples of massecuites, M.L. was collected after 5, 30, and 60 minutes. The sucrose and invert sugars concentration were determined, and the data are presented in Table 2.

Table 2.--Mother liquor (50 g sample)<sup>1</sup>

Spin time (min)	Sucrose, % <sup>2</sup>	Invert sugars, % <sup>2</sup>
5	51.6	9.1
30	51.1	9.2
60	51.9	8.1

<sup>1</sup> Average of duplicate determinations

<sup>2</sup> HPLC analysis

The results indicate that no differences exist between the first portion of M.L. removed and the total composite sample removed after 60 minutes. On the basis of these findings it can be concluded that there is no need to make a complete separation of M.L. and crystals to determine yield by weight since a representative can be obtained in 5-10 minutes. The small change in concentration because of the cooling effect on large samples during extended spinning times is therefore not a problem.

#### Application to continuous vacuum pan

Crystals and M.L. separations were made from magma samples taken from different sections of a continuous vacuum pan operation. The M.L. was analyzed for sucrose, invert sugars, ash and color. The magma samples were random samples taken at different times and on different days. No attempt was made to monitor the change in sucrose and invert sugars for a given batch of massecuite, although ideally this would be the best and most useful approach. The data for sucrose, invert and ash are presented in Table 3. As expected, not only does the sucrose concentration of the M.L. decrease and the invert sugars concentrations increase, there is the expected increase in ash content. These results are better illustrated in Figure 3. Again, it should be pointed out that the data are for random samples which would account in part for the variations noted. The line drawn represented the ideal situation and in this instance the slope of the line would indicate the efficiency of the operation.



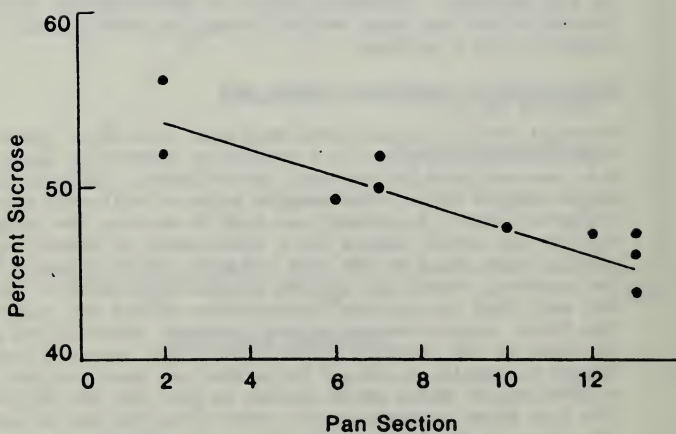
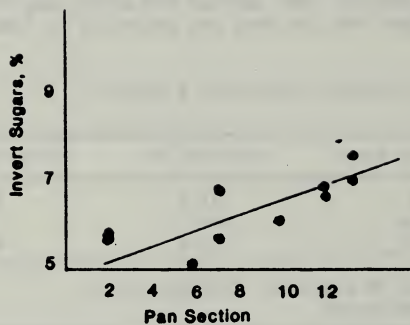


Figure 3.--Magma from Continuous Vacuum Pan.

Table 3.--Mother liquor from continuous vacuum pan operation<sup>1</sup>

Pan section	Sucrose, % <sup>2</sup>		Invert sugars, % <sup>2</sup>		Ash, %	
	set 1	set 2	set 1	set 2	set 1	set 2
2	56.1	52.1	5.7	5.7	7.4	7.6
7	51.8	50.0	5.7	6.8	9.1	8.3
13	47.4	48.9	6.9	7.0	7.9	9.8
	set 3		set 3		set 3	
6	49.2		5.1		6.8	
10	47.6		6.1		9.0	
12	47.2		6.8		10.8	
13	46.1		7.6		10.5	

<sup>1</sup> Random samples<sup>2</sup> Determined by HPLC analyses

Color measurements indicated intensity differences were notable among massecuites run on different days and among the various pan sections.

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## DISCUSSION

Question: In the graph where you show time versus sucrose content, you can see that the more time, the lower the sucrose content. The sucrose grain is like a magnet. It attracts sucrose, and the molasses closest to the growing grain has the lowest sucrose content.

Bailey: That was true of the 200g sample. In the 50g sample, we showed that the first portion that was removed had the same composition as the composite removed after one hour.



